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A STUDY OF THE RELATION BETWEEN ACTUAL AND NORMAL YIELDS OF IMMATURE DOUGLAS FIR FORESTS[1]

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INTRODUCTION

For the several methods of predicting the yields of even-aged forests that for years have been much discussed, the yield of normal or fully stocked forests has been generally accepted as forming the most desirable basis. For this purpose normal-yield tables are worked up showing the volume of wood produced by a fully stocked forest on any growing site at any age of its life. A fully stocked forest, at least as defined in the Douglas fir region, is one in which the canopy is fairly complete and average maximum cubic-foot volumes are obtained. The growing space, in other words, is well utilized and the volumes are as large as can be expected on the average in unthinned stands.

The first difficulty that comes up when a forest manager or a landowner wishes to apply normal-yield tables to a particular piece of land for the purpose of predicting the volume at some future period is that the actual, or "empirical," stand is not in a normal condition throughout, and therefore normal-yield tables can not be used without a reduction of their values. He may say that since the values must be reduced the yield tables should be based on average, not normal, conditions. A very little observation will lead anyone to conclude that even the actual condition is far from constant. One tract may be heavily stocked, another lightly stocked, or a single tract may have several degrees of stocking. No empirical table would be apt to fit actual cases without appropriate allowances.

Since many forests of Douglas fir have approximately complete canopies, no trouble would be experienced in locating small fully stocked or normal sample plots scattered throughout almost every tract, but over larger areas there are always a number of irregularities. A fire may have gone through, killing a few trees and causing small openings; a windstorm may have broken tops or uprooted trees; insects may have killed small patches here and there; creeks and low wet places may cause breaks in the canopy; steep slopes and precipices may interrupt the stand; or reproduction may not have been successful from the very beginning. In other

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words, a variety of factors, accidental, physiographical, and physiological, contribute to the departure from a normal condition. The usefulness of a normal table as a standard of reference, representing, so to speak, 100 per cent conditions, becomes more and more striking the further one considers the question.

If this concept of normal-yield tables is adopted, such significant questions as the following must be answered:

What is the relation between the actual and the normal forest?
Does this relation change during the life of the stand?
What causes and what constitutes understocking or overstocking?
How is degree of stocking recognized?
What methods of procedure are best when normal tables are to be used to make yield predictions of a forest?

The American literature on the application of normal-yield tables is not complete. Certain rules for judging stocking and predicting growth, based chiefly on European methods, have been followed and are stated in most standard books on forest mensuration and regulation. (Graves, Chapman, Nisbet, Roth, Woolsey.) But no literature can be found covering adequately the relation between the actual stand and the normal stand, nor any investigations which test the soundness of the methods of application that are advocated. Some light upon this phase of the subject may be obtained from studies recently pursued in the Douglas fir region by the Pacific Northwest Forest Experiment Station.

In 1925 a study of normal yields of second-growth Douglas fir (*Pseudotsuga taxifolia*) was completed in Oregon and Washington for the region lying west of the Cascade Range.[2] During 1926 and 1927, after the normal yield tables for Douglas fir had been completed, a further field study in understocked and overstocked stands was made. This work attempted to answer questions regarding the application of normal tables to actual stands and the relation between normal and empirical stands. It is with this second study that the present paper is concerned.

As used in these Douglas fir yield studies the term "normal" forest, defined as representing a fully stocked condition, allows a certain flexibility in density in regard to both number of trees and volume. In the construction of yield tables this variation is taken into account by allowing a certain range in the basic data. Averages from these ranges are referred to as the 100 per cent, or normal, yields, and all other yields are then expressed in terms of this standard. "Actual" forests are defined as stands actually found over large areas. "Understocked" is the term usually applied to a stand that is poorer than normal, and "overstocked" to one in better than normal condition. These words, however, are not precise, since, as already stated, normal plots in themselves cover a rather wide range of stocking.

The material gathered in the course of the study is divided into three classes. The first class consists of the records taken on 5,014 chains of strip survey, 785 of which were made by R. E. McArdle

[2] McArdle, R. E. THE YIELD OF DOUGLAS FIR IN THE PACIFIC NORTHWEST. Tech. Bul. 201. 56 p., illus. 1930. McArdle, R. E. RATE OF GROWTH OF DOUGLAS FIR FORESTS. STANDARD YIELD TABLES FOR FULLY STOCKED SECOND-GROWTH STANDS. West Coast Lumberman 54: 90–95, illus. 1928.

in 1924 and 1925 and the rest by the writer in 1926 and 1927. The second class consists of 493 sample plots selected from the basic data of the normal-yield study, of which 433 were used to cover the complete range of age and site conditions and 118 plots of the 70-year age class alone were used to analyze the effect of eliminating the variable of age. The third class of material consists of an actual yield survey covering 4,190 acres, of which 2,631 acres are classed as being in the immature Douglas fir type. Very little will be said of this last phase of the study, since it pertains to questions outside the scope of this paper.

The 5,014 chains of strip survey were located in 83 different representative second-growth Douglas fir forests. The strips were practically all 1 chain (66 feet) wide; in a few very dense stands the width was reduced to 0.5 chain. Every tree on the strip was calipered, and a constant check was kept upon the age by taking increment borings to the centers of average-sized dominant trees. The average height of the dominant and codominant trees was used as an index of site quality. When differences in age or site class were found, new tally sheets were started. The sheets were also changed after every 10 chains or full-acre tally. The width of the strip was checked by pacing out 33 feet on each side of the tape. A small range finder set to 33 feet probably would be quite satisfactory for this purpose and would show at a glance from the position of the images whether a tree should be included or not. Continuous notes were kept concerning the slope, the character of the stocking, the size, and probable cause of gaps in the Douglas fir canopy.

In the second season's work only the trees of the main canopy were tallied, the understory trees and the hardwoods being left out altogether. In the first season the dead trees were counted and tallied in two classes—old and recently dead—in an attempt to find out whether an advance to a normal condition was indicated by the rate of mortality. The underlying idea was that if an understocked stand were approaching a normal condition, the loss by mortality would be less than in a normal forest. This idea was put to test immediately after the first field season and found to be of no value. Therefore, the tally of the dead trees was dropped in the succeeding season.

The second class of material consists of detailed records taken on temporary sample plots, all rectangular in shape, varying from 0.25 to 1 acre in size. In the normal-yield study the plots were combined by the tracts in which they were taken, and a composite acre was computed for each tract. By this procedure many of the minor variations from acre to acre were disguised. Recomputation of the single plots was deemed necessary for an accurate comparison of the degree of stocking on strips compared to the degree of stocking on picked acres or plots.[3]

[3] The details of the temporary sample plot measurements and of their combination into a normal yield table are described in full by McArdle. (See footnote 2.)

RELATION BETWEEN ACTUAL STANDS AND NORMAL STANDS

COMPUTATIONS

Mathematical computations are required to show the extent of the difference between the actual forest and the normal stand—how wide a range of stocking may be expected, how large a factor is the presence of small holes in the canopy, and what relation the stand values bear to one another. For this purpose a number of the processes known to graphical and statistical analysis [4] were applied to the first two classes of material. Only the more important relations appear in this text, since many of the computations failed to be significant.

The data are treated both as individual strip acres and as tract averages. To obtain a measure which would put all sites and ages

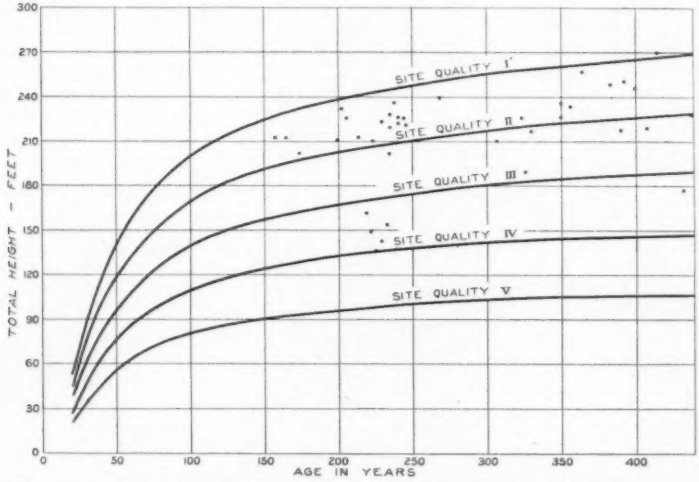

FIGURE 1.—Average heights of dominant and codominant Douglas firs to be used in classifying lands by quality of site

on a comparable basis, all the values had to be expressed in terms, or rather percentages, of the normal-yield table values, called variously "degrees of stocking," "normality percentages," "stocking percentages," and so forth. For instance, if a tally shows a total of 260 trees to the acre and a total basal area of 255 square feet on a site and at an age for which the normal values are 300 trees and 280 square feet, the number-of-trees normality percentage is $260 \div 300$, or 87 per cent, and the basal-area normality percentage is $255 \div 280$, or 91 per cent. Similar normality percentages were obtained for (1)

[4] BRUCE, D., and REINEKE, L. H. CORRELATION ALINEMENT CHARTS IN FOREST RESEARCH. A METHOD OF SOLVING PROBLEMS IN CURVILINEAR MULTIPLE CORRELATION. U. S. Dept. Agr. Tech. Bul. 210, 1930. [In press.]

SMITH, B. B. THE USE OF PUNCH AND TABULATING EQUIPMENT IN MULTIPLE CORRELATION PROBLEMS. U. S. Dept. Agr., Bur. Agr. Econ. 1923. [Multigraphed.]

SMITH, B. B. CORRELATION THEORY AND METHOD APPLIED TO AGRICULTURAL RESEARCH. U. S. Dept. Agr., Bur. Agr. Econ. 1926. [Multigraphed.]

number of trees 11.6 inches d. b. h.[5] (12-inch class) and larger, (2) total cubic-foot volume, (3) board-foot volume by International rule with ⅛-inch kerf, and (4) board-foot volume by Scribner rule, as well as for (5) the total number of trees and (6) total basal area.

TABLE 1.—*Normal yield tables for Douglas fir*[a]

SITE QUALITY I

Total age (years)	Stand		Total basal area[b]	Average d. b. h.		Average height dominant and co-dominant trees	Volume[c]		
	All trees	12 inches d. b. h. and over		All trees	12 inches d. b. h. and over		Total stand	International rule	Scribner rule
	Number	*Number*	*Sq. feet*	*Inches*	*Inches*	*Feet*	*Cubic feet*	*Board feet*	*Board feet*
20	571	7	101	5.7	12.6	52	1,830	4,900	400
30	350	57	154	9.0	13.7	92	4,750	24,400	8,000
40	240	109	195	12.2	15.2	120	7,500	47,000	24,400
50	176	126	224	15.3	17.1	139	10,150	70,500	44,100
60	138	118	248	18.2	19.2	156	12,500	90,800	62,000
70	113	105	268	20.9	21.4	170	14,500	108,500	78,200
80	97	93	285	23.3	23.6	181	16,350	124,700	92,500
90	84	84	299	25.6	25.6	192	17,880	137,700	104,800
100	75	75	312	27.6	27.6	200	19,140	148,900	115,100
110	69	69	323	29.4	29.4	207	20,200	157,900	123,700
120	63	63	332	31.1	31.1	213	21,090	165,500	131,100
130	59	59	341	32.7	32.7	218	21,840	172,000	137,700
140	55	55	350	34.3	34.3	221	22,520	178,000	143,500
150	51	51	357	35.8	35.8	224	23,170	183,300	148,700
160	48	48	364	37.2	37.2	226	23,780	188,100	153,500

SITE QUALITY II

20	880		98	4.5		44	1,550	2,100	
30	555	27	150	7.0	13.0	78	4,110	16,000	2,600
40	385	75	189	9.4	13.9	102	6,550	34,900	11,900
50	290	120	217	11.8	15.0	119	8,840	55,000	27,400
60	228	141	241	14.0	16.2	132	10,860	72,800	42,800
70	186	140	260	16.0	17.7	144	12,660	89,000	57,200
80	159	133	276	17.9	19.0	154	14,220	103,200	70,000
90	138	124	290	19.6	20.4	163	15,540	114,700	81,000
100	123	115	302	21.2	21.8	170	16,610	124,400	90,400
110	111	106	313	22.6	23.1	176	17,560	133,000	98,300
120	101	99	322	24.0	24.2	181	18,340	140,300	105,100
130	94	93	331	25.3	25.4	185	19,000	146,500	111,000
140	88	87	338	26.5	26.6	188	19,590	152,000	116,300
150	82	82	346	27.7	27.8	190	20,130	156,700	121,200
160	78	78	353	28.9	28.9	192	20,650	161,100	125,700

SITE QUALITY III

20	1,460		92	3.4		37	1,250	200	
30	865	6	140	5.5	12.5	64	3,300	8,400	300
40	585	37	177	7.4	13.1	84	5,250	22,000	4,500
50	430	79	204	9.3	13.9	98	7,050	37,100	12,400
60	337	118	226	11.1	14.7	109	8,700	52,000	23,800
70	274	139	244	12.8	15.6	119	10,150	65,000	35,200
80	232	148	259	14.3	16.5	127	11,350	77,200	45,700
90	204	149	272	15.6	17.4	134	12,390	86,700	55,000
100	184	145	283	16.9	18.4	140	13,270	94,700	62,800
110	166	139	292	18.0	19.2	145	14,000	101,500	69,400
120	152	134	301	19.1	20.0	149	14,600	107,200	75,000
130	141	128	309	20.1	20.8	152	15,140	112,200	80,000
140	131	123	317	21.1	21.6	154	15,610	116,900	84,500
150	123	117	324	22.0	22.4	156	16,080	121,100	88,600
160	117	113	331	22.8	23.1	158	16,490	125,000	92,400

[a] Adapted from tables by R. E. McArdle. (See footnote 2.)

[b] Total basal area includes all trees of the stand.

[c] Cubic volume includes the total cubic-foot volume inside bark of all the trees in the stand. Board-foot volume by international rule for ⅛-inch kerf includes the volume of all trees in the 7-inch diameter class and larger (actually 6.6 inches plus) to a 5-inch top inside bark. Board-foot volume by Scribner rule includes the volume of all trees in the 12-inch class and larger (actually 11.6 inches plus) to an 8-inch top inside bark.

[5] D. b. h.=diameter at breast height, or 4.5 feet above average ground level.

TABLE. 1—*Normal yield tables for Douglas fir*—Continued

SITE QUALITY IV

Total age (years)	Stand		Total basal area	Average d. b. h.		Average height dominant and co-dominant trees	Volume		
	All trees	12 inches d. b. h. and over		All trees	12 inches d. b. h. and over		Total stand	International rule	Scribner rule
	Number	*Number*	*Sq. feet*	*Inches*	*Inches*	*Feet*	*Cubic feet*	*Board feet*	*Board feet*
20	3,069		81	2.2		29	870		
30	1,472		122	3.9		50	2,270	2,400	
40	927	7	153	5.5	12.6	66	3,560	9,200	200
50	659	29	177	7.0	13.1	77	4,780	19,000	3,300
60	500	58	195	8.5	13.6	86	5,880	28,900	8,100
70	405	90	211	9.8	14.1	94	6,830	37,900	14,000
80	345	114	224	10.9	14.6	100	7,690	45,700	20,100
90	304	130	235	11.9	15.1	105	8,400	52,200	26,000
100	271	141	245	12.8	15.7	110	9,000	58,100	31,400
110	247	146	254	13.7	16.2	114	9,500	63,200	36,300
120	224	149	261	14.6	16.7	117	9,920	67,500	40,700
130	209	150	268	15.3	17.2	119	10,290	71,000	44,700
140	195	149	275	16.0	17.7	121	10,620	74,300	48,300
150	184	147	281	16.7	18.1	123	10,920	77,500	51,600
160	175	144	287	17.4	18.6	124	11,200	80,100	54,600

SITE QUALITY V

20	6,920		64	1.3		21	520		
30	2,700		96	2.6		37	1,330		
40	1,530		121	3.8		48	2,110	1,500	
50	1,050	1	140	4.9	12.4	56	2,840	5,900	30
60	780	12	154	6.0	12.7	63	3,500	10,500	1,100
70	625	27	166	7.0	13.0	68	4,090	15,400	2,400
80	525	44	177	7.9	13.3	73	4,580	20,300	4,400
90	451	62	185	8.7	13.6	77	5,000	24,900	6,900
100	403	78	193	9.4	13.9	80	5,350	28,800	9,600
110	362	93	200	10.1	14.2	83	5,640	32,000	12,200
120	331	105	206	10.7	14.5	85	5,900	34,700	14,700
130	305	114	213	11.3	14.8	87	6,130	37,000	17,000
140	284	122	218	11.9	15.0	88	6,340	39,200	19,200
150	266	127	223	12.4	15.3	89	6,520	41,300	21,300
160	250	132	227	12.9	15.6	90	6,670	43,300	23,300

A generalized yield table (Table 1) presents the essential normal-stand values. In the computations a more detailed table was used. Instead of the five broad site-quality classes shown in the table, actual site-index classes were used. Site index is an indicator of site, deduced from the average height of the dominant and co-dominant trees when the stand is 100 years of age. A set of curves similar to Figure 1 relates the height at any age to the height the stand will have at 100 years. Site-index classes of Douglas fir range from 80 to 210 feet. In Figure 1,[6] Site I represents site index 200; Site II, site index 170; Site III, site index 140; Site IV, site index 110; Site V, site index 80. Table 1 lists three volume measurements—cubic-foot volume (the total cubic-foot volume inside bark of all the trees); board-foot volume by International rule for ⅛-inch kerf, which includes only trees of the 7-inch d. b. h. class and larger; and board-foot volume by Scribner rule, which includes only trees of the 12-inch d. b. h. class and larger.

[6] Up to the 160-year point this graph is based upon the very large number of trees whose heights were measured in the course of the normal-yield study. In order that it might be possible to determine the site-quality class of land still covered with old-growth forests, additional measurements were made, in 40 different even-aged stands from 150 to over 400 years old, of the average height of the dominant and codominant trees, and the height-on-age curves for the five principal site-quality classes were projected.

AVERAGE DEGREES OF STOCKING

The average values of the empirical stands will be considered first and compared to the normal-yield tables. Strip samples in many second-growth Douglas fir forests gave a good idea of how much, on the average, natural forests underrun the normal. The large bare areas, or surveyable openings, were left out, and only the small holes or gaps in the Douglas fir canopy, at the most a couple of chains across, which are always found and can not be surveyed out, were included in the strip area. Table 2 lists the average relation of these actual stands to normality by five classes of data, namely, by strip samples for the first season and for both seasons, by combined tract tallies, and by two classes of selected sample plots in normal stands.

TABLE 2.—*Average degrees of normality of actual stands of immature Douglas fir and of selected sample plots*

Class of data	Stand		Total basal area	Volume		
	All trees	12 inches d. b. h. and over		Cubic measure	International rule	Scribner rule
	Number	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Strips (1926); 179 acres; average age, 82.8 years; average site index, 162	82.4	85.5	89.4	89.2	92.3	93.7
Strips (1926 and 1927); 531 acres; average age, 77 years; average site index, 159	77.4	77.5	80.7	80.5	82.1	83.3
Tracts (1926 and 1927); 81 tracts	78.2	79.5	82.0	82.1	84.3	85.7
Sample plots from normal yield study; all ages; 433 plots; average 77.4 years; average site index, 155	103.4	105.5	107.0	109.7	----------	111.5
Sample plots from normal yield study; 70-year age class; 118 plots; average age, 75 years; average site index 150	99.7	95.7	99.2	102.6	----------	99.7

In the preparation of Table 2 a number of erratic values were rejected. One tract, for instance, was thrown out because it was obvious from the character of the averages that either the age or the site had been determined wrongly. Number of trees decreases, but basal area and volume increase with advance in age or improvement in site. The effect of an error in age or site determination upon the percentages of normal number of trees will therefore be exactly the opposite to the effect upon the percentages of normal basal area or volume. If, for instance, the age is overestimated, the computed percentage of normal number of trees is increased and the percentage of normal basal area is decreased. Therefore, in a study of this nature, extreme accuracy in the determination of age and site is essential. Certain strip-acres whose actual or normal board-foot volume was zero were also disregarded—temporarily, at least.

Table 2 is the source of much interesting information. It is remarkable that the average value of the tracts sampled is so high in degree of stocking. Based upon the total strip acres measured in both years, the average stand has 77 per cent of the normal number of trees, 81 per cent of the normal basal area, 80 per cent of the normal cubic-foot volume, and 82 to 83 per cent of the normal board-foot volume. These values are all appreciably lower than those

based on the first season's work alone, for the reason that in the first season the better stands were visited, and in the second season the study was extended to the poorer forests.

It must be emphasized that these averages do not apply to extensive, diversified areas, unless the meadows, barren land, other forest types, etc., are surveyed and excluded from consideration. In a survey of 4,190 acres, of which 3,700 acres were actually covered by strips, only 2,631 acres were classed as second-growth Douglas fir type. The remaining area consisted of other types, such as hardwoods (202 acres), cedar, hemlock, and white pine types (422 acres), major waterways and river flats (531 acres), and veteran stands (404 acres). Such large surveyable eliminations must be given consideration in dealing with any extensive tract.

RANGE IN DEGREE OF STOCKING

The average of Table 2 will apply in general, but individual acres and tracts may vary widely from them. To illustrate to what extent actual values can vary, there are listed in Table 3 the maximum and

TABLE 3.—*Standard deviations and actual (not theoretical) maxima and minima of the values composing the averages shown in Table 2 given to indicate the range of values to be expected*

Factor	Strips, 1926, standard deviation	Strips, 1926 and 1927			Temporary sample plots			
					All ages			70-year class, standard deviation
		Standard deviation	Total range	Range of central two-thirds of cases	Standard deviation	Total range	Range of central two-thirds of cases	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Total number of trees	26.1	24.9	14–179	52.5–102.3	29.5	40–250	73.9–132.9	26.6
Trees 12 inches d. b. h. and larger	21.6	26.2	.....	51.3–103.7	41.5	.....	64.0–147.0	25.6
Basal area	19.5	19.4	25–145	61.3–100.1	18.7	60–170	88.3–125.7	15.7
Cubic-foot volume	19.4	19.7	25–138	60.8–100.2	22.0	60–210	87.7–131.7	22.2
International board-foot volume	20.5	21.2	25–144	60.9–103.3	.....	.....	.....	.....
Scribner board-foot volume	22.8	28.0	9–286	55.3–111.3	57.6	0–550	53.9–163.1	29.5

minimum percentages of normal stocking that were found and the standard deviations from the averages. The standard deviation represents a range on each side of the average in which approximately two-thirds of the variations can be expected to be confined. For instance the standard deviation of basal area for the 1926 and 1927 strips is 19.4 per cent; its corresponding average from Table 2 is 80.7 per cent. Then in two-thirds of the instances the differences between the individual strip acre and the average lie between 80.7 plus 19.4 and 80.7 minus 19.4, or between 100.1 and 61.3 per cent. Theoretically all cases should lie between the average minus and the average plus three times the standard deviation or, for 1926-27 basal area, between 22.5 and 138.9 per cent. The actual minimum and maximum according to Table 3 are 25 and 145 per cent.

The variability for basal area, cubic-foot volume, and International board-foot volume, as shown by the percentage standard

deviation, is surprisingly uniform. However, number of trees and Scribner board-foot volume are distinctly more variable and show these factors to be subject to different influences.

There are two reasons for the increased variability of the Scribner volume. The first is that only a part of the stand is considered. The increase in number of trees 12 inches and more d. b. h. is comparatively rapid at the age when stands are getting their first 12-inch trees, and there are apt to be large percentile differences between the actual stand and the yield-table values, since a few trees can have great effect upon the total board-foot volume. Therefore, young stands or stands on poor sites can be expected to show these erratic percentages in Scribner board-foot volume. Because of this, difficulties can be expected when the values in basal area, cubic-foot volume, etc., are related to the Scribner values.

The second reason is that the Scribner rule does not accurately measure the lumber which can be produced from logs and trees and that this inaccuracy is greatest in small trees. Part of the variability which is being discussed is therefore undoubtedly due merely to errors inherent in the rule used.

Scribner rule, not being a precise unit of volume measure and being applied only to the merchantable trees, does not lend itself to analytical treatment, and almost invariably it proves itself unamenable to precise methods. Were it not for the fact that board-foot rule is a current commercial unit for expressing stand volume, it would be preferable to omit it entirely. Therefore, if undue emphasis seems to be placed upon it here, that is because much effort was spent in trying to get expressions of stand values related to this conventional unit of measure.

The failure of the percentages of number of trees to be in line with the percentages of the other factors is explained by the numerous small understory trees or persistent suppressed trees in some stands, but not in all. The effect of this variation is to render this factor useless.

These ranges in stocking percentages of strip acres are apparently large, but they are exceeded in certain cases by the ranges in stocking of square sample acres. The variation in number of trees on 433 plots is between 40 and 250 per cent, with a standard deviation of 29.5 per cent. This is very interesting in view of the fact that all the plots were chosen for their fairly normal and homogeneous appearance. Basal area shows a range from 60 to 170 per cent with a standard deviation of 18.7 per cent and cubic-foot volume a range from 60 to 210 per cent, with a standard deviation of 22 per cent. Scribner board-foot volume is entirely out of proportion, as might be expected.

If whole tract averages are taken (not listed in Table 3) instead of the strip-acre averages, the ranges are reduced somewhat, for then they vary only from 38 to 152 per cent, for number of trees, 48 to 125 per cent for basal area, 51 to 122 per cent for cubic-foot volume, and 52 to 130 per cent and 30 to 219 per cent, respectively, for international and Scribner board-foot volumes.

In this study occasional individual strip acres and possibly more frequent rectangular sample plots were actually the tallies of portions of acres put on the acre basis. As a rule plots computed upon the

acre basis give higher averages and larger deviations. In the author's opinion this does not explain away, however, the large differences which exist between strip acres and plots and does not invalidate the statement that sampling an area by strips is more reliable than by scattered plots, since it automatically covers the slight irregularities which occur continually in a stand and which are not smoothed out so effectively if square plots are taken. As a rule the error of estimate increases with the perimeter, and strips have the larger perimeter for a definite area. However, the errors of a carefully conducted survey nowhere nearly approach the errors caused by the variability of the stand. The whole is a controversial subject and can be answered finally only by actual test.

SMALL HOLES AND THEIR EFFECT UPON STOCKING

The difference between actual stocking and normal stocking is due to certain causes. First of all, the breaks in the stand, the gaps in

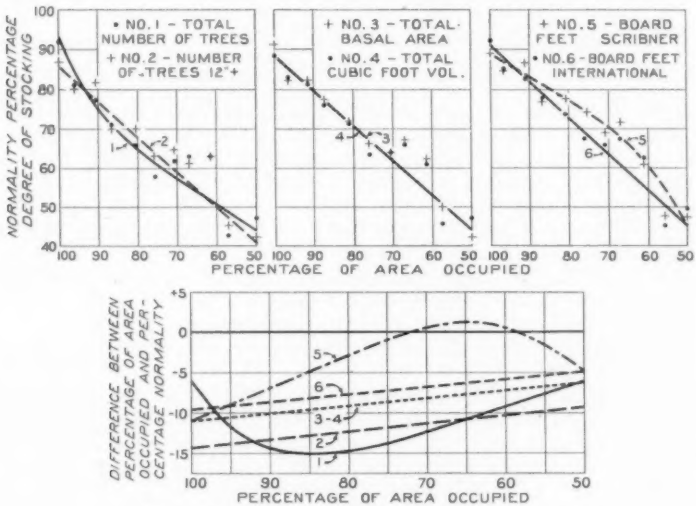
• NO. 1 - TOTAL NUMBER OF TREES
+ NO. 2 - NUMBER OF TREES 12"+
+ NO. 3 - TOTAL BASAL AREA
• NO. 4 - TOTAL CUBIC FOOT VOL.
+ NO. 5 - BOARD FEET SCRIBNER
• NO. 6 - BOARD FEET INTERNATIONAL
NORMALITY PERCENTAGE DEGREE OF STOCKING
PERCENTAGE OF AREA OCCUPIED
DIFFERENCE BETWEEN PERCENTAGE OF AREA OCCUPIED AND PERCENTAGE NORMALITY
PERCENTAGE OF AREA OCCUPIED

FIGURE 2.—Effect of area occupied upon stocking

the canopy, or the proportion of the total area in small holes should be examined to see whether understocking consists chiefly in wide spacing of trees or in numerous small openings. The small holes or breaks in the second-growth canopy of 2 chains or less may be occupied by no trees at all, by hardwoods, by other conifers, or by veterans. The character of the stand and the size of the holes were noted on 442 strip acres; 132 of the strip acres had no portion of the area in holes, 95 had 1 to 5 per cent of their area in holes, 78 had 6 to 10 per cent, 52 had 11 to 15 per cent, and so forth, as shown in Table 4. By plotting the average stocking percentages for each group over the percentage of area occupied (lower half of fig. 2), the roughly curved values of Table 4 were obtained. When less than 70 per cent of the area is occupied, the points are apt to be erratic because of the scarcity of data.

TABLE 4.—*Relation between area occupied and stocking*

Area occupied [a]		Curved normality percentage					
Per cent	Acres	Number of trees	Basal area	Number of trees 12 inches and over	Volume		
					Cubic measure	International rule	Scribner rule
100	132	94	89	86	89	91	89
95	95	83	84	81	84	86	86
90	78	75	80	77	80	82	83
85	52	70	75	72	75	77	80
80	29	65	71	68	71	72	77
75	17	61	66	63	66	68	74
70	11	58	62	59	62	63	71
65	10	54	57	54	57	59	66
60	5	51	53	50	53	54	61
55	4	47	48	45	48	50	54
50	0	44	44	41	44	45	45

[a] 9 acres were less than 50 per cent occupied, of which 5 were less than 45 per cent.

Each one of the factors (Table 4) maintains with only minor variations a similar relation between occupied area and stocking. They all start a trifle below 100 per cent and stay within 13 per cent of each other the whole way through. In other words, the actual small openings seem to play the greatest part in the variation in understocking, while the spacing of the trees plays a less important and also less obvious rôle. Speaking generally, there seems to exist approximately a 10 per cent difference between the degree of stocking and the area occupied.

The lower part of Figure 2 shows in another way the effect of holes upon the degree of stocking. In this chart the difference between the stocking percentage and the percentage of the area occupied is plotted over the percentage of area occupied. The trends of the curves for basal area, cubic foot, International rule, and number of trees 12 inches and more are fairly parallel, rectilinear, and slowly rising. The other two curves, for total number of trees and Scribner-rule volume, are distinctly different. What plausible explanations, if any, exist for these relationships, and what light do they throw upon the character of the understocking? In the first place the gross effect of holes is eliminated and the lesser effect of tree spacing is made apparent. A low proportion of the area in small holes means a great dropping off in the total number of trees. However, since the trend in the percentage of 12-inch trees is rising, it is apparent that the trees chiefly affected are below this diameter limit, or are those of the poorer dominance classes. Increased frequency of holes, within certain limits, indicates greater canopy space for the remaining trees and hence better individual development, or, in other words, a larger proportionate number of 12-inch trees and, furthermore, a greater proportionate Scribner volume. The Scribner curve and number-of-trees curve are of similar shape but exactly inverse in position with regard to both coordinates.

CAUSES OF THE SMALL HOLES IN A FOREST

Undoubtedly the character of the stocking in relation to the area occupied depends largely upon the original cause and age of the hole. Unfortunately such relations are practically indeterminable, and all the investigator can do in a short-time study is to show the relative importance of the several agencies. For this purpose field observations were made of the probable origin of each hole. Table 5 summarizes these, dividing them into size groups and estimating the measure of their respective importance by giving an approximation of the total area covered. Out of 660 holes, 17 per cent are listed as "unknown." "Veterans" include small areas eliminated because they are occupied by veteran trees. "Fire," "insects," and "wind" holes are apparents from the present condition of the trees or stand. "Gullies and creeks" combine often with "hardwoods or wet places" where Douglas fir establishment is seldom a success and where cedar and hemlock or hardwoods take its place. "Rock outcrops and steep slopes" are sometimes contributing factors to unevenness in stocking. "Natural" holes are those apparently due to original failure in stocking, where no Douglas fir ever started.

The holes below 0.05 acre are by far the most numerous, amounting to two-thirds of the total number, although they aggregate only about one-third of the total area. By and large, 9.5 per cent of the average acre is in small holes. This estimate was obtained by an independent summing of the sizes of the holes and not from the rough classes of Table 5.

TABLE 5.—*Cause and size of small holes in immature Douglas fir stands* [a]

Cause or nature of holes	Small holes, classified by size of hole as indicated							Approximate total area	
	0.0–0.049 acre	0.05–0.099 acre	0.1–0.149 acre	0.15–0.199 acre	0.2 acre and over	All holes			
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Acres*	*Per cent*
Fire	35	8	2	1		46	7	1.91	6
Veterans	45	13	5			63	10	2.72	9
Insects	34	9	1			44	7	1.65	5
Wind	62	14	4			80	12	3.10	10
Gullies and creeks	51	30	7	2		90	28	4.76	37
Hardwoods or wet places	44	33	12	7	1	97		6.58	
Rock outcrops and steep slopes	25	4	2	1		32	5	.66	2
Natural	43	14	6	4		67	10	3.58	12
Unknown	87	21	4	1		113	17	4.44	15
Miscellaneous	19	9				28	4	1.16	4
Total	445	155	43	16	1	660	100	30.58	100

[a] Of the 63 tracts from which these data are taken, 29 show traces of fire, 28 show no traces, and 6 are doubtful.

Gullies and creeks plus hardwoods form by far the most important combined factor both in number of holes and in area. Together they constitute 28 per cent of the holes and 37 per cent of the total area in holes. They even slightly outrank in number the sum of natural and unknown holes. Wind is the next most important factor with 12 per cent in number of cases and 10 per cent in area. Presence of veterans in the stands (10 per cent of the holes) ranks next, with damage by fire (7 per cent) and insects (7 per cent) following.

Other minor causes include rock outcrops and steep slopes (5 per cent), and miscellaneous (4 per cent), which include small cuttings and roads.

The fact that fire ranks so low in causing small holes is noteworthy, especially since 29 tracts out of 63 described show clear traces of fire. Despite its pronounced thinning agency in much younger stands than those studied and its importance as a destroyer of whole forest stands, fire is not a prominent cause of small holes. Clean burns caused by crown fires, not the light surface burns which thin out young stands, are a prevalent cause of extensive gaps in a forest. Failure of man-made openings to restock properly is another. Both are of overwhelming significance but do not find much place in the present discussion on understocking of stands. The character and quantity of such openings are different for each locality and average values would have no utility. In this study the strip-survey lines were stopped when such openings were encountered.

EFFECT OF UNDERSTOCKING OR OVERSTOCKING ON METHOD OF YIELD PREDICTION

An important part of this study is to determine the actual effect of understocking or overstocking upon such stand values as number of trees, basal area, or volume. The analysis will give an idea of the best index of stocking and its relation to other values. If basal area is found to be a reliable index of stocking, will cubic-foot volume or board-foot volume have exactly the same normality percentages as basal area? If not, what significance can be attached to the difference, and what effect will this difference have on yield predictions? The chosen factor should be relatively simple so that reliable indications of volume normality will be obtained with the minimum of computation.

Stocking, or the degree of normal condition, can be expressed in terms of basal area, number of trees, or any of the volume values. Ordinarily one considers a heavily stocked forest one in which the trees are so close together as to form a complete, dense canopy. The other definitions of stocking deal with secondarily derived factors—for instance, with basal area, for which a diameter tally is needed, and with volume, for which a height curve in addition to the diameter tally is necessary. Undoubtedly an index based upon volume itself is most accurate. However, with volume taken in its broad sense, the relation would vary greatly, depending chiefly upon the product for which the stand is being raised—whether for pulp wood, for ties, for poles, for boards, or for timbers. Many of these expressions of stocking in terms of merchantable products are of transitory value only and can easily be omitted from consideration.

The method of testing whether number of trees or basal area is the better index of stocking may be based first upon a curve of stocking in percentages of volume, averaged in 10 per cent classes, over number of trees or basal area. A smooth logical trend will be an evidence of relationship. Statistical measures will be computed, such as the correlation coefficients and the standard errors, the regression lines will be plotted, and certain correlation indices and modified standard errors will be found. In this way the relationships which give the best-fitting regression lines or modified curves, as evidenced

by high correlation coefficients or indices and low standard errors, will be those from which the most suitable index of stocking is obtained.

Correlations

Table 6, presenting standard deviations and correlation and alienation coefficients for the surveyed strips, facilities further reference and avoids undue repetition. The correlation coefficient is an indication of straight-line relationship between two variables. Complete association is signified by 1, therefore the closer a coefficient is to 1 the better is the relation. The alienation coefficient, on the other hand, measures the actual proportion of variation attributable to other causes, including curvature of the relation between the two in question.

Table 6.—*Standard deviations, correlation coefficients, and alienation coefficients computed for surveyed strips in actual stands*

Factor	Average stocking	Standard deviation	Relation [a]	Number of trees 12 inches and over	Basal area	Volume		
						Cubic measure	International rule	Scribner rule
	P. ct.	*P. ct.*						
Number of trees	77.4	24.9	C. C.	0.377±.025	0.613±.018	0.577±.020	0.473±.023	0.120±.029
			A. C.	(.926)	(.790)	(.817)	(.881)	(.993)
Number of trees 12 inches and over in diameter.	77.5	26.2	C. C.		.656±.017	.662±.016	.749±.013	.847±.008
			A. C.		(.755)	(.750)	(.663)	(.532)
Basal area	80.7	19.4	C. C.			.955±.003	.926±.004	.685±.016
			A. C.			(.297)	(.378)	(.729)
Volume, cubic feet	80.5	19.7	C. C.				.935±.004	.658±.017
			A. C.				(.355)	(.753)
Volume, International rule.	82.1	21.2	C. C.					.832±.009
			A. C.					(.555)
Volume, Scribner rule	83.3	28.0						

[a] The correlation coefficient (C. C.) is given plus or minus the probable error of the coefficient, which is $0.6745\frac{1-r^2}{\sqrt{n}}$ (n being 531). The alienation coefficient (in parenthesis) equals $\sqrt{1-r^2}$, where r is the correlation coefficient. Alienation coefficient (A. C.) $=\frac{\text{SE}=\text{SD about curve}}{\text{SD}=\text{SD about mean}}$.

Number of Trees as an Index of Stocking

First let the criterion of number of trees be examined, since it would be the simplest index if workable. All the values have been previously converted to percentages of the normal yield table values. In Figure 3 the average points of each 10 per cent group are plotted over the percentages of number of trees as abscissas, and the regression lines (mathematically computed lines of average trend) are drawn through them. If the several plottings are superposed they show very similar trends. The points indicate a sharp rise from 0, passing between 60 and 80 per cent values at 40 per cent normal number of trees, then increasing gradually to a maximum of 100 to 110 per cent at about 120 per cent normal number of trees, after which there is an apparent dropping off. The data beyond 120 per cent, however, are too few for accurate conclusions.

Trends of this nature indicate that, in general, volume per cent at first rises rapidly with an increasing relative number of trees,

soon slows up, reaches a maximum, and then falls off with further increase in number of trees. In other words, there seems to be an ideal number of trees for maximum volume. This number, moreover, being at least 120 per cent, strongly suggests that the conception of normality involved in the yield tables is somewhat conservative. However, graphical representation alone is insufficient and statistical measures must be employed to show whether the relations are trustworthy.

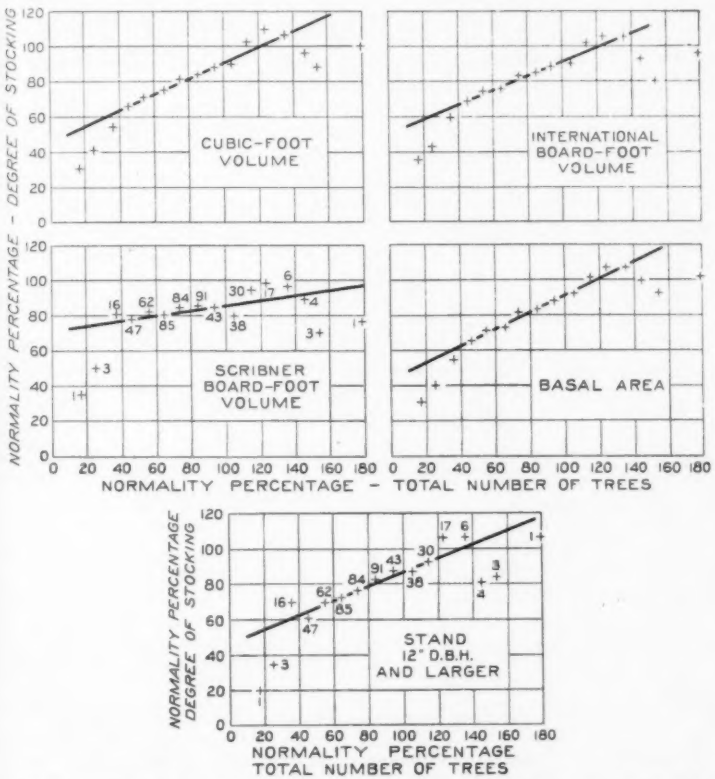
NORMALITY PERCENTAGE - DEGREE OF STOCKING
CUBIC-FOOT VOLUME
INTERNATIONAL BOARD-FOOT VOLUME
SCRIBNER BOARD-FOOT VOLUME
BASAL AREA
NORMALITY PERCENTAGE - TOTAL NUMBER OF TREES
NORMALITY PERCENTAGE DEGREE OF STOCKING
STAND 12" D.B.H. AND LARGER
NORMALITY PERCENTAGE TOTAL NUMBER OF TREES

FIGURE 3.—Degree of stocking with respect to volume and number of trees 12 inches and larger, related to total number of trees as an index of stocking. Straight lines represent computed regression lines

The correlation coefficients (Table 6) indicate very well how weak the relations are. The coefficients between degree or normality of number of trees and number of trees 12 inches in diameter and larger, basal area, cubic-foot volume, international board-foot volume, and Scribner board-foot volume are 0.377, 0.613, 0.577, 0.473, and 0.120, respectively. The plus or minus quantities after the coefficients of Table 6, indicating the probable errors of the coefficients, are in every case very small and negligible. The respective

alienation coefficients are 0.926, 0.790, 0.817, 0.881, and 0.993, all of which are percentages of variation due to other factors and to curvature if present, as explained previously. Because of the small number of weights in the portions of the average curves which deviate in a pronounced manner from the regression line, for instance from 0 to 30 per cent and from 130 to 180 per cent, the correlation indices of the fitted curves would probably be improved by less than 0.10 increase. Correlation index is to a fitted curve what the correlation coefficient is to the regression line. The number-of-trees relation with Scribner volume is amazingly weak, altogether too weak for further use, although one might reason that decrease in number of trees would be accompanied by increased size of the individual trees, hence increased board-foot volume.

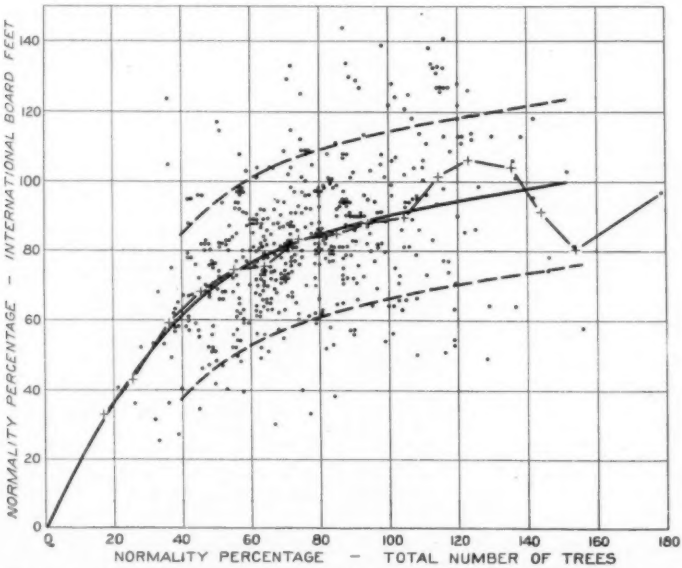

FIGURE 4.—Dispersion of normality percentages of International board-foot volume with normality percentage of total number of trees as a basis for stocking

By taking the coefficient between total number of trees and volume by International rule (0.473) and reproducing the actual points in addition to the average values of Figure 3, an array such as that presented in Figure 4 is obtained. This bears out graphically the contention that the correlation coefficient could not be improved very much by introducing curvature to the straight-line regression. The band of points is very wide, and there would be little benefit in any further attempts to use the percentage of normal number of trees as an index of stocking, especially since from Table 6 it is very apparent that other relations exist which have much greater value. In a preliminary test of a portion of the data (1926 data), improvement of the number-of-trees relation to Scribner volume was sought by

fitting a curve, but the correlation index showed an increase of only 0.03 over the correlation coefficient for the straight line regressional.

An additional method of showing the weakness of the number-of-trees percentage in relation to International-volume percentages of Figure 4 is to compute the standard error around the curve, plot it, and notice how wide a belt it forms. This is done assuming that the correlation coefficient is not much improved by drawing the curve. The alienation coefficient for the correlation coefficient 0.473 is 0.88. Since the alienation coefficient is equal to the standard error over the standard deviation $\left(AC=\frac{SE}{SD}\right)$ and the standard deviation for international volume is 24.9 per cent, the standard error is 21.9 per cent. Actually standard error is a little smaller than this, depending upon the improvement made by passing the curve through the points instead of using the regression line. The broken-line curves on either side of the average curve represent the range in which two-thirds of the cases can be expected to lie.

Why should total number of trees show such a poor connection with stocking? The explanations undoubtedly are that it is hard to eliminate the trees which do not really belong to the main stand, and do not comprise most of the basal area and volume, that the trees are unevenly spaced, and that many relatively small trees persist or do not persist. These irregularities affect the percentages of number of trees but do not appreciably affect the percentages of basal area or volume.

Basal Area as an Index of Stocking

Basal area is the next factor to be considered, since the simple factor of total number of trees fails to be significant. The stocking percentages for board-foot volume by Scribner rule and by International rule and for total cubic-foot volume are plotted upon the stocking percentages for basal area. (Fig. 5.) The plottings of all three follow the same trend, remaining within 5 per cent of each other practically throughout. The 45° angle which they make with the axes affords a very favorable trend for prediction purposes. Indicated curvilinearity is very slight. Consequently the improvements to the correlation coefficients of Table 6 would be small if curves were fitted. The correlation coefficients for International board-foot volume and for cubic-foot volume with respect to basal area are high, but even a small improvement would make the relations more perfect. Board-foot volume by Scribner rule again seems to be more erratic, with the result that basal area is not as good an index of Scribner stocking as desired. This weakness will undoubtedly be a source of error in making yield predictions for board-foot volume by Scribner rule by means of stocking percentage founded on basal area. The cubic-foot volume predictions and the International board-foot volume predictions will, however, be much more reliable. The coarse-scale graphs (fig. 4) do not permit determining the relationships with sufficient accuracy. A table of values read from the original large-scale figures giving the best curved values, not necessarily the regression values of Figure 5, is drawn for further use. (Table 7.)

TABLE 7.—*Relation of the normality percentages for board-foot volume and cubic-foot volume with basal area as index of stocking*

Basal area	Cubic-foot volume normality	Board-foot volume normality by—		Basal area	Cubic-foot volume normality	Board-foot volume normality by—	
		International rule	Scribner rule			International rule	Scribner rule
Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
10	10	10	8	110	109	110	113
20	20	20	19	120	118	119	122
30	30	30	30	130	127	128	131
40	40	40	41	140	136	136	140
50	50	51	52	Correlation coefficient	0.955	0.926	0.685
60	60	61	62	Alienation coefficient	.297	.378	.729
70	70	71	73	Standard error	5.9	8.0	20.4
80	80	81	83				
90	90	91	92				
100	100	101	103				

The alienation coefficients for basal area with respect to volume in cubic feet, International board feet, and Scribner board feet are 0.297, 0.378, and 0.729, respectively. The corresponding standard

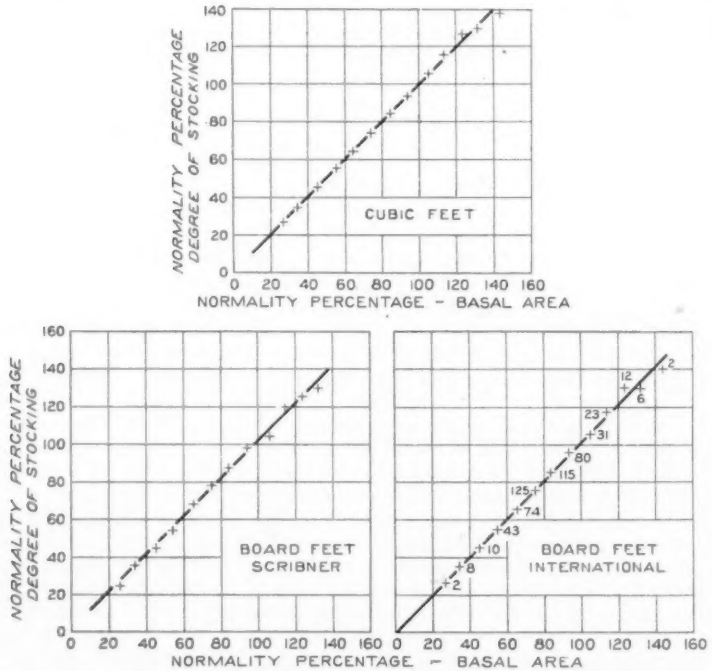

FIGURE 5.—Degrees of stocking with respect to cubic-foot volume and board-foot volume related to basal area as an index to stocking. Straight lines represent computed regression lines

errors are stated in Table 7 as 5.9, 8, 20.4 per cent. The 8 per cent error makes a very favorable contrast to the 21.9 per cent that was

obtained when total number of trees was used as an index for identical board-foot volume. The Scribner relation, although it forms a nice line of averages (fig. 5), has not been so favorable a standard error.

Basal area, therefore, with its good correlation coefficients in respect to cubic-foot volume and International board-foot volume, its resulting low standard errors, and its convenient form of relationship (a 45° angle), is satisfactory. If a better index for Scribner board-foot volume is still desired, other factors must be examined.

Number of Trees 12 Inches and over as Index of Board-foot Volume

Throughout the computations those dealing with board-foot volumes by Scribner rule are always the most variable, contain the most erratic values, and are the most unsatisfactory to analyze for consistent, valuable relations. So far it has been shown how poorly both total number of trees and basal area are correlated with Scribner-rule volume. In seeking another stronger relationship it must be remembered that estimates of board-foot volume of small timber by the Scribner rule are misleading unless accompanied by an estimate of the overrun percentage. If this could be determined and applied, a volume in board feet would result which would differ little from the volume by the International rule, but, as a matter of fact, this overrun percentage is itself very variable. For those wishing the Scribner volume, the best indicator proves to be the percentage of normal number of trees 11.6 inches and more d. b. h., since, according to Table 6, the correlation coefficient between the two is 0.847.

Figure 6 shows graphically that a certain degree of curvilinearity exists. The erratic points beyond 140 per cent, being of little use, are eliminated. They represent chiefly values read from the relatively weak portions of the yield tables—values for the low ages or the poor sites, where number of 12-inch trees is small and volume by Scribner rule is slight, and where any small exceptional condition would disturb the percentages greatly. Setting the maximum stocking percentage for number of 12-inch trees at 140 per cent will reduce the correlation coefficient for Scribner volume from 0.847 to 0.800, but will increase that for cubic-foot volume from 0.662 to 0.728 and that for International volume from 0.749 to 0.787. It is evident that a curve will express the relation between number of 12-inch trees and volume by Scribner rule much more accurately than the regression lines, especially in the low and high percentages where the plotted points deviate pronouncedly from the regression line. Table 8 gives these readings, together with a separate set of readings from a curve drawn in exactly similar fashion except that the individual acres are first grouped and are averaged by the tracts in which they were taken.

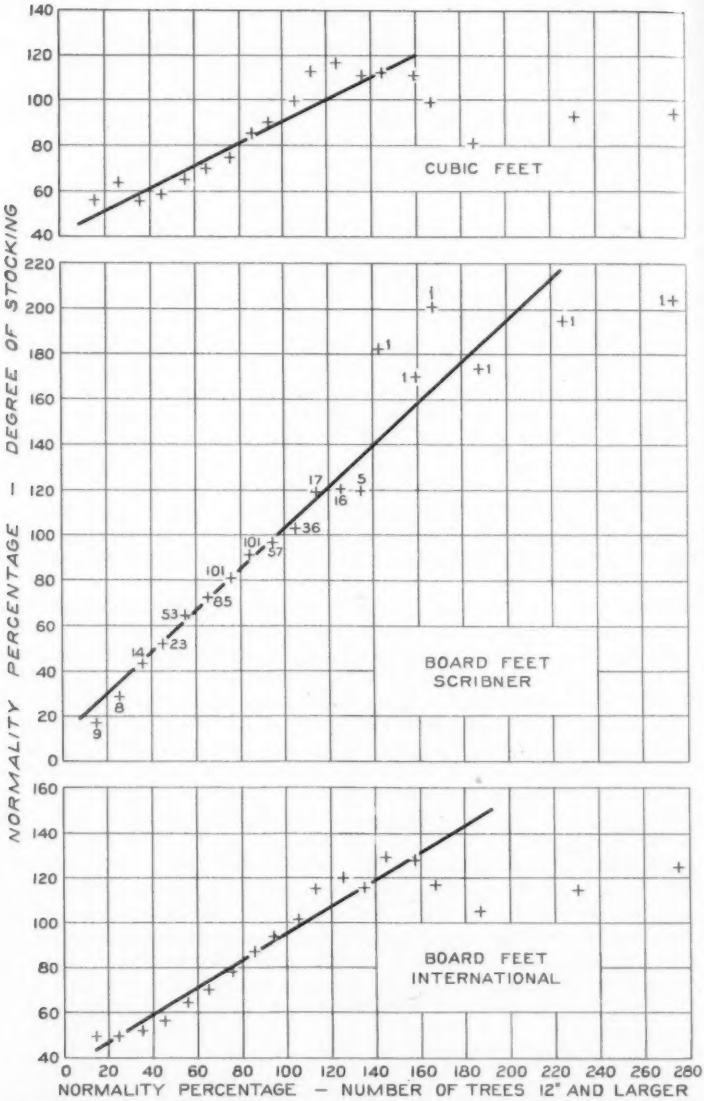
CUBIC FEET
BOARD FEET SCRIBNER
BOARD FEET INTERNATIONAL
NORMALITY PERCENTAGE - DEGREE OF STOCKING
NORMALITY PERCENTAGE — NUMBER OF TREES 12" AND LARGER

FIGURE 6.—Degrees of stocking with respect to volume related to number of trees 12 inches and larger as an index of stocking. Straight lines represent computed regression lines

TABLE 8.—*Relation of the normality percentages for board-foot volume by Scribner rule with number of trees in the 12-inch d. b. h. class and larger as index of stocking*

Number of trees 12 inches and larger	Volume normality in board feet, Scribner rule on—		Number of trees 12 inches and larger	Volume normality in board feet, Scribner rule on—	
	Individual strip acres	Tracts		Individual strip acres	Tracts
Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
10	10		100	103	101
20	22		110	111	108
30	34	30	120	118	115
40	46	44	130	125	121
50	58	56	140	132	128
60	68	67	Correlation coefficient	0.800	0.771
70	78	77	Correlation index	.837	.838
80	87	85	Alienation index	.547	.546
90	95	93	Standard error	13.7	11.8

For the main portion of the curves between 50 and 100 per cent the differences between the two sets are not at all striking. The inconsistency in the extremes is easily explained by the small number of tracts having average values within these ranges. This relation for board-foot volume by Scribner rule is easily applied and practical, especially since many of the cruises in second-growth Douglas fir are being made to a minimum diameter limit of 12 inches. The fact should be noted that the volume percentages overrun percentages of the number of 12-inch trees in the main range by 3 to 8 per cent. Due corrections must be made for this overrun in computing the yield predictions where this index of stocking is used.

The number of trees in the 12-inch class obviously can not be used as an index of stocking in young stands. It should be used only after there is a fair proportion of trees of this size, probably 50 per cent or more, and then with much caution and with the full understanding that results are not absolutely accurate and that there still exists a large chance for error in individual cases. With any other species except one which attains large sizes in relatively short time, it will be of no use at all. The best that can be said of this relation is that it is the closest found in all those tested. Why should an index involving number of trees 12 inches and larger be usable when total number of trees gives no reliable indication? It is because the variable number of small trees of the understory and in thickets of suppressed growth below 12 inches d. b. h. are eliminated by a 12-inch diameter limit and only trees with a merchantable volume are included.

RELIABILITY OF THE INDICES OF STOCKING

The dependability of these two chosen indices of stocking, basal area, and number of 12-inch trees is indicated in another way by stating the amount of error which is liable to occur in using the relations for single acres. This error, the standard error, has been mentioned before. The method of determining it corresponds to the method of computing the standard deviation except that the

regression line or fitted curve is used as a base instead of the mean of the data. It is most easily computed from the correlation coefficient and standard deviation by means of this formula—

Standard error = standard deviation × the square root of (1 − correlation coefficient squared), or—

$$SE = SD\sqrt{1-CC^2}.$$

Tables exist for the quantity under the radical sign.[7]

A range plus and minus of one standard error from the curve value includes about two-thirds of all the possible cases that are likely to be found; a range plus and minus of twice the standard error includes 95 per cent of all the cases. In Table 8 it is seen that the standard error for the relation of stocking in percentage of number of trees 12 inches and larger to volume by Scribner rule is 13.7 per cent. If number of trees is used as the base, the standard error for Scribner volume percentages will be 27.8, and if basal area is used it will be 20.4 per cent (see Table 7), showing that of these three relations the first is by far the most valuable. The relation between basal area and cubic-foot volume has a standard error of 5.9 per cent, and that between basal area and volume by international rule has one of 8 per cent; both of these are comparatively small and indicate a close relationship. With total number of trees as the basis, similar standard errors for cubic-foot volume and International board-foot volume are two to three times as great (16.1 and 18.7 per cent).

An instance of the significance of these standard errors may be cited. If on a single acre the total basal area is 60 per cent of the normal for that particular site and age, then the percentage of cubic-foot volume in two-thirds of all expected cases will lie between approximately 54 per cent and 66 per cent of the normal yield table value (60 ± 5.9), or, in other words, for a single instance there is a two out of three chance that it will be within 5.9 per cent of 60 per cent. In case the total number of trees 12 inches and larger is 60 per cent of the normal, then in two-thirds of all cases the normality percentage of board-foot volume by Scribner rule will lie roughly between 54 per cent and 82 per cent; according to Table 8, 60 per cent in number of 12-inch trees corresponds to 68 ± 13.7 per cent of Scribner volume.

These ranges of values apply to single acres only; the more acres taken the smaller will be the expected total error. For instance, if on 1 acre the standard error is 5.9 per cent, then on 100 acres of tally the standard deviation of the average will be only $\sqrt{\frac{.059}{100}}$ or approximately 0.6 per cent. Thus, if the 60 per cent normal basal area is an average of 100 strip acres, then there are two chances in three that this average is within 0.6 per cent of the true average cubic-foot normality of the 100 acres.

[7] MINER, J. R. TABLES OF $\sqrt{1-(r)^2}$ AND $1-(r)^2$ FOR USE IN PARTIAL CORRELATION AND IN TRIGONOMETRY. 49 p. Baltimore, Md. 1922.

Relation of Other Factors to Board-Foot Volume by Scribner Rule

After the original data had been compiled and analyzed, and after it was seen how good an index to total cubic-foot volume and International board-foot volume basal area was, and how relatively good an index to Scribner board-foot volume the number of trees in the 12-inch d. b. h. class and larger was, it was suggested that the basal area of such trees might be a still better index. The suggestion is undoubtedly valuable and this index should be tested at some future time.

It is extremely unfortunate that no more certain index of volume in board feet by Scribner rule was discovered. One suggestion is that the use of two factors instead of one would help. Work was done along this line with the 433 sample acres taken in the course of McArdle's yield study. The strongest multiple correlation coefficient of three factors, of which Scribner volume was one, involved total number of trees, number of trees 12 inches and larger, and, of course, board-foot volume by Scribner rule. The coefficient of correlation amounted to 0.899. Methods of curvilinear multiple correlation were applied as far as the third approximation, and the improvement in the correlation coefficient was then tested. The new correlation index amounted to 0.904, hardly an appreciable improvement. Further manipulation evidently would not produce more certain results, and the attempt was dropped at this stage. Even omission of the erratic values did not help much. The coefficient of 0.904 expresses, of course, a close relation; but, since the original standard deviation for this material was extraordinarily high (57.6 per cent, see Table 3), the final standard error was still too large for practical use.

Another test was made to determine what factors really influence Scribner volume. The underlying idea is based upon partial correlation in which the relation between two factors is first studied without considering the effect others may have; then other factors which may have some effect are introduced one at a time, and the change in the correlation coefficient is noted. A pronounced change indicates that the introduced factor plays some part in the relation. When the relation between the stocking percentages of number of 12-inch trees, board-foot volume by Scribner rule, total number of trees, and total basal area are taken in this way, the results of the computation are as listed in Table 9.

Table 9.—*Partial coefficients of correlation showing the effect of basal area, total number of trees, and number of trees 12 inches and larger, upon board-foot volume by Scribner rule*

Correlation 0 order		Correlation first order		Correlation second order
Scribner, number of trees	0.120	With 12-inch trees constant	−0.4048	With both constant	−0.7109
		With basal area constant	−0.5210	
Scribner, basal area	0.685	With number of trees constant	0.7794	With both constant	0.6583
		With 12-inch trees constant	0.3225	
Scribner, number of trees 12 inches and over	0.847	With number of trees constant	0.8720	With both constant	0.8224
		With basal area constant	0.7230	

Four factors, composing three pairs, are considered. In each pair one of the other factors is introduced by assuming it to be held constant while the original two are allowed to vary. Thus the original relation between Scribner volume and total number of trees is expressed by the coefficient of 0.120. If the number of 12-inch trees be held constant, then the coefficient changes to −0.4048; in other words, if the number of 12-inch trees remains the same then the percentages of Scribner volume varies inversely with the total number of trees; if the basal area is held constant, a similar effect is obtained. By holding both constant the reasonably large coefficient of −0.7109 is obtained. Another pair of factors, basal area and volume by Scribner rule, shows an increase if the total number of trees be held constant (a decrease if the number of 12-inch trees be held constant). That the two neutralize each other is seen from the final coefficient. The relation between the third pair, Scribner volume and number of 12-inch trees, is found to be fairly independent of the other two factors, as is evident from the fact that the coefficient is only slightly affected by holding one or the other factor constant. This in itself argues for the adoption of such a criterion for judging the normality of board-foot volume by Scribner rule, because it furnishes an independent relation.

It is recommended, in making short-term yield predictions, that basal area be used as index of stocking for cubic-foot volume and International board-foot volume, and that the normality of number of trees 12 inches and larger be used as index for Scribner board-foot volume. In long-term predictions, however, basal area should be used as the index for all classes of volume.

ADVANCE FROM UNDERSTOCKING TO A MORE NORMAL CONDITION

Up to this point no reference has been made to the possibility that the degree of normality might change with the passage of time, especially if stocking is based upon total number of trees. Many persons have believed that the more open the stand—that is, the fewer the trees—the smaller would be the loss in numbers through mortality and the better would be the individual growth of the trees; stands would become nearly normal in character if sufficient time elapsed. Also, it was urged, the fewer the trees and the better the individual development the relatively larger would be the percentages of basal area and volume with respect to the percentage of total number of trees. This condition in itself could be taken as evidence that the stands were approaching normality. Based upon such an argument, Figure 3 would indicate that approach to normality was a very real condition if the dispersion of the plots around the average curve were not considered. But, as explained before, each curve really represents a wide belt from which, if appropriate choice be made, any theory can be proved. It has been shown that an index to degree of normality must be referenced to basal area or number of 12-inch trees rather than total number of trees.

Very little can be said about the changes with passage of time in the normality percentage of number of trees 12 inches and larger. If a stand has an unduly large number of 12-inch trees, it may maintain this advantage for at least a number of years.

More direct observations upon the change which is likely to take place in an understocked forest have been obtained in the study of growth on permanent sample plots,[8] some of which have been under observation for 15 years. Because of the small number of plots (12), the percentages are somewhat erratic. When estimated in terms of basal area the rise in degree of stocking of understocked plots in a 5-year period is 2.7 per cent; with cubic-foot volume, 4.5 per cent; with volume by Scribner rule, 2 per cent. This is so small and uncertain a correction, and is so overshadowed by larger and more important variables, that it may often be most practicable to ignore it.

Since this percentage is small, always erratic, always affected by the least irregularity in mortality rate, and unreliable in its computation even on permanent sample plots, it is certain that not much progress can be made in determining advance toward normality by studies of plots or strips measured but once. In such studies past or future diameters and height curves must be computed or drawn, allowances must be made for mortality, or whole diameter groups must be dropped out entirely to compensate for mortality.

There are a sufficient number of permanent plots in coniferous forests which have been measured for several periods and from which, by relatively little study, a good idea can be obtained of the approach to normal condition with the passage of time. In Douglas fir at least 32 plots are being measured periodically and more are being added each year.

ANALYSIS OF TEMPORARY SAMPLE PLOTS

Chiefly for academic rather than practical interest, this study was extended beyond the method of analyzing strip samples to that of analyzing plot samples. A set of 433 plots was chosen from the normal yield-study data. These plots were selected to represent equally all age classes and all site classes. In order to eliminate the effect of age, a set of 118 plots in the 70-year age class was used.

EFFECT OF SLOPE UPON DEGREE OF STOCKING

A strip system of sampling can not give information on the effect of slope because there is too much variation. On single plots, however, a certain degree of slope can be chosen and the effect of slope on stocking can be effectively defined. Figure 7 gives graphically the results of this portion of the study, and Table 10 lists the readings corrected for the average—that is to say, since the average basal area, cubic-foot volume, and Scribner board-foot volume for the 433 plots amounted to 107 per cent, 109.7 per cent, and 111.5 per cent, respectively, of the normal values, the readings of the curves had to be reduced by an amount equal to 7, 9.7, and 11.5 per cent, respectively. These degrees of overstocking of a random choice of 435 plots probably resulted from the elimination of irregular plots, which would tend to raise the averages.

[8] MEYER, W. H. RATES OF GROWTH OF IMMATURE DOUGLAS FIR AS SHOWN BY PERMANENT SAMPLE PLOTS. Jour. Agr. Research 36: 193–215, illus. 1928.

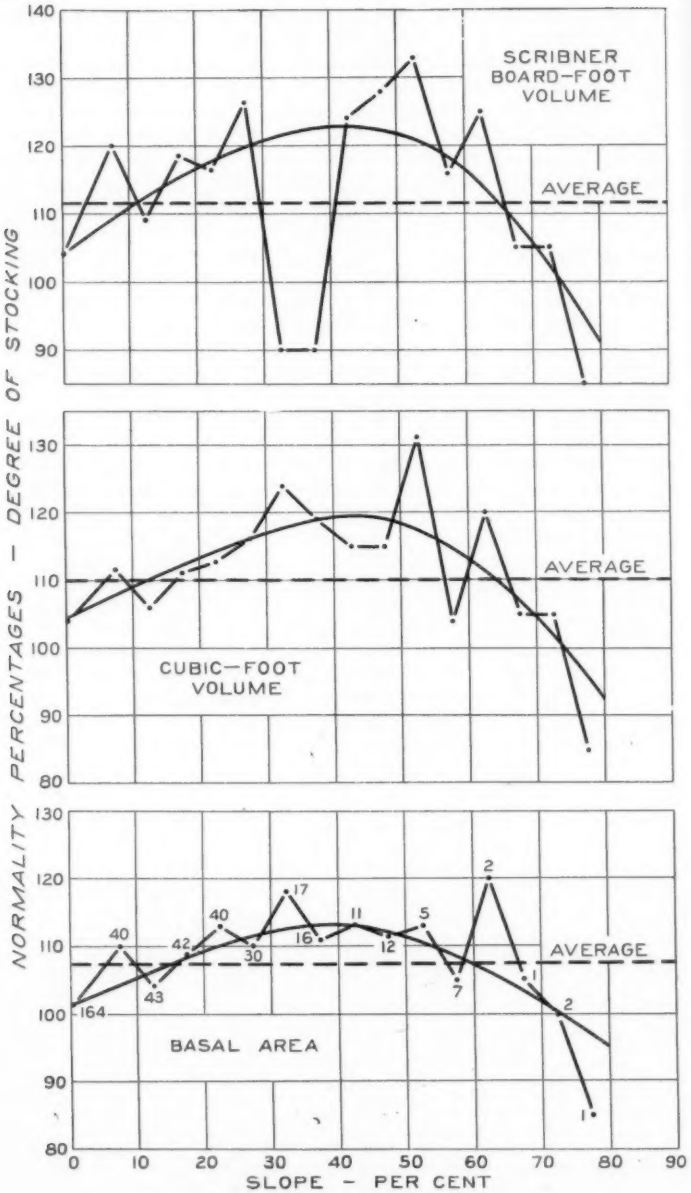

FIGURE 7.—Effect of slope upon degree of stocking

TABLE 10.—*Effect of slope upon the degree of stocking*

Slope	Correction			Basis, plots	Slope	Correction			Basis, plots
	Basal area	Volume				Basal area	Volume		
		Cubic feet	Board feet (Scribner)				Cubic feet	Board feet (Scribner)	
Per cent	*Per cent*	*Per cent*	*Per cent*	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Number*
0	94	95	92	164	50	104	108	111	17
10	98.5	99.5	99	83	60	100	103	105	9
20	102	103.5	104.5	82	70	95	94.5	94	3
30	105	107	109	47	80	88	82.5	80	3
40	106	109.5	111.5	27					

According to Table 10 the maximum yields are produced on slopes of 40 per cent. Even if the acres were transposed to surface measure instead of using horizontal measure, as is done throughout this study, slopes of about 30 per cent would still be indicated as producing a maximum yield. The percentages have been found to be conservative; values for the 70-year class, for example, are still larger. From the shape of the curves, low correlation coefficients can be expected. A much improved correlation index should be obtained if a curve is fitted to the plotted points. This test was applied to the 118 plots of the 70-year-age class. The correlation coefficient between slope percentage and basal-area normality was only 0.186, but the correlation index of the fitted curve was 0.922, a great improvement. The correlation coefficient between slope percentage and Scribner-volume normality was 0.307; the correlation index was 0.460. Much closer relationships than are indicated by these coefficients probably exist, but they are obscured by the wide range of stocking in the original material.

The explanation of this effect of slope upon the yield probably lies in the increased amount of light the individual tree receives as a result of the "staggering" of the tops. The significance of the effect of the slope on stocking is somewhat veiled by the varied topography on many areas.

EFFECT OF ASPECT UPON DEGREE OF STOCKING

Intimately connected with the question of slope is that of aspect. McArdle,[9] in his study of normal yields, concludes that the best yields are obtained on north and east slopes. No very definite conclusions could be reached in the course of strip surveys, since a strip can cover a number of changes in aspect. However, general observations gave definite support to McArdle's statement. On the steeper south and west slopes, at least, the site qualities are continually changing with each break in topography, each draw, each scabby ridge. The past events in the history of the area surely have had some effect; these hotter slopes may have been burned more fiercely and have had a greater impoverishment of their soil than the moister north and east slopes. In critical situations a successful restocking can not be expected, since the surface soils dry out too quickly after the cessation of the winter and spring rains.

[9] See footnote 2.

EFFECT OF SITE UPON DEGREE OF STOCKING

The effect of site upon the stocking of a stand has proved quite indeterminate, although all groups of data were subjected to analysis. If the poorer sites have lower degrees of stocking, the data do not show it. The reader must keep in mind, however, that the discussion is still of normality percentages and not of absolute values.

COMPARISON OF PLOT METHOD WITH STRIP METHOD

A very interesting comparison is that of the relations of stocking obtained by strip analysis with those obtained by plot analysis. The preparation of tables similar to Tables 6, 7, and 8, and figures similar to Figures 3, 4, 5, and 6, although it led to no new conclusions, brought out at least one astonishing and disturbing condition—the increased variability of some of the relations and the poorer correlations. One might expect that picked plots would exhibit less variability, since they are chosen as well-stocked areas; but actually a strip automatically irons out many inconsistencies that are maintained in a square plot of the same acreage. The size of the plots also has something to do with their variability, since small plots put on the acre basis will show a greater range of values and sometimes higher averages than full-acre plots. In this study, however, no plots are below 0.25 acre in size and a great many are 0.5 to 1 acre. The standard deviation of basal area remains about the same, being (according to Table 3) 19.4 for the strips, 18.7 for the 43 plots, and 15.7 for the 118 plots of the 70-year-age class. The widest difference occurs in the normality percentages of board-foot volume by Scribner rule. The 433 plots have a standard deviation of 57.6 per cent as compared to 28 for the strips and 29.5 for age class 70. The young forests and the poor sites, where Scribner volumes are small and chance for percentage errors large, are partially the cause of the difference.

In their essential points the relations between stand factors are surprisingly alike, whether computed by strips or by plots. The more material taken, the more uniform will the results be. Based upon the strip surveys taken during the first season alone, certain differences were noticed and significance attached thereto, but after the second season's investigations were added the differences were ironed out and lost all significance. For example, with regard to the dependence of normality of board-foot volume by Scribner rule upon that of number of trees 12 inches and larger, the final analysis of the strips gave a regression equation of the following form—

Scribner volume per cent=0.905 (percentage of trees 12 inches and over)+13.2.

To this is compared the regression obtained by taking 433 sample plots—

Scribner volume per cent=0.930 (percentage of trees 12 inches and over)+9.9

or that of the 118 plots of the 70-year-age class—

Scribner volume per cent=0.864 (percentage of trees 12 inches and over)+ 17.1.

This agreement is remarkable considering that three distinct groups

of material are treated. When curved over the actual points the agreement is still more perfect.

The relation between normality of total cubic-foot volume and that of total basal area is not quite so good, but still the differences are not pronounced enough to overthrow any conclusions.

For strip surveys: Cubic-foot volume per cent=0.972 (basal area per cent)+2.1.

For 433 plots, all ages: Cubic-foot volume per cent=0.917 (basal area per cent)+10.4.

For 118 plots, 70-year class: Cubic-foot volume per cent=1.145 (basal area per cent)—10.2.

There is nothing so disturbing, therefore, that we can not adopt the values of Tables 7 and 8 in the actual practice of yield application on an extensive scale. However, on small local areas the variation can be as wide as the limits prescribed by the standard errors of the curves.

QUALITY OF WOOD, AS WELL AS QUANTITY, AFFECTED BY STOCKING

The result of understocking upon the quality of the lumber produced is very important and is strikingly shown in Figure 8, but quality of wood in the tree is difficult to appraise. A study of quality growth not only must be distinct from the study of quantitative production, but it involves many additional technical difficulties and a large number of tests of the quality of logs or lumber produced in normal stands as well as in understocked stands. Observation shows that open-grown stands produce coarser-grained wood, more knots, and larger knots, but no qualitative data are available as to the actual quality differences between the product of normal stands and that of understocked stands. This is a question of prime importance and an attempt should be made to solve it, probably by cutting acres of different degrees of stocking, determining the grade or quality class of products yielded by actual conversion to merchantable units, and attempting to arrive at workable conclusions.

SUMMARY

Normal-yield tables are chiefly valuable as a standard for judging the future yield of existing forest stands. But it is well known that actual forests on extensive areas differ appreciably from the normal condition and by a degree which is not constant but is distinct for each separate area. Some forests may be very much understocked, some only slightly so, and some may even be overstocked. In order to use normal-yield tables to predict future yields of stands of all these degrees of stocking, it is necessary to know the fundamental relation between such stand factors as number of trees, basal area, and volume as affected by degree of stocking. Since degree of stocking must be defined in terms of volume, which is a value requiring much computation, it is desirable to have as simple a measure as possible for an index of stocking. This index would be used in the field for recognizing the degree of normality.

The relations found in this study between the normal yield table stand values and the stand values of actual forests concern only quantitative wood production. They do not take into consideration

FIGURE 8.—Single stands may show great variations in stocking. These two photographs were taken within a few hundred yards of each other. In a very widely spaced portion of the stand (B) the trees became overdeveloped and limby; where conditions were somewhat above normal, as in A, trees were of good form but had a much smaller average diameter than in the first area

utilization of stands nor quality production, both most important but distinct phases of forest-yield predictions.

Some of the significant findings of this study may be summarized as follows:

To arrive at the real acreage of the type under consideration, the areas of all large openings and of other types must be mapped out and eliminated from consideration. Small holes and gaps are left in. In the Douglas fir canopy these small gaps amount on an average to 10 per cent of the total area. The most important cause of such gaps in this type is not fire, but the presence of gullies, creeks, and small hardwood patches.

Understocking is primarily attributable to the presence of small holes, and is only secondarily a factor of wider spacing.

Second-growth stands of Douglas fir, not including the major openings and gaps, approximate slightly better than 80 per cent of the normal-yield table values, this estimate being based on the results of strip surveys in 83 individual tracts.

Total number of trees is a very unsatisfactory index of total volume stocking; basal area is the best and most reliable index of volumes in cubic feet and board feet by the International rule. For board-foot volume by Scribner rule the number of trees 12 inches d. b. h. and larger is more reliable.

It is recommended that for short-term predictions basal area be the index for cubic-foot and International board-foot volume stocking, and that number of trees 12 inches and larger be the index for board-foot volume by Scribner rule. For long-term predictions basal area should be the index of stocking for all three units of volume.

Very little regarding the advance of an understocked stand to a more fully stocked, or normal, condition was learned from the analysis of these once-measured plots and strips, but periodic measurements of permanent plots indicate that this advance goes approximately at the rate of 4 per cent every decade for stands between 40 and 80 years of age.

The highest volumes are obtained on approximately 40 per cent slopes, referenced to horizontally measured acres, site for site. South and west aspects are apt to be quite variable in site. North and east aspects are the more favorable ones. Site apparently has no effect upon the average degree of stocking.

Sampling a tract by strips is considered more reliable than by plots, provided strip width be properly controlled. But the relation between pairs of stand factors, such as basal area, cubic-foot volume, and board-foot volume, are much alike whether determined by strip surveys or by sample plots.

STUDIES OF VACCINATION DURING CALFHOOD TO PREVENT BOVINE INFECTIOUS ABORTION[1]

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HISTORICAL REVIEW

More than 30 years have elapsed since Bang announced his notable discovery with reference to the etiology of bovine infectious abortion. During this period an immense amount of work has been performed by different investigators in endeavors to improve methods of abortion prevention and control. In many cases the investigations reported have naturally dealt with immunization procedures. Data pertaining to this phase of the abortion problem have been contributed by Bang (*1*),[3] McFadyean and Stockman (*10*), Zwick and his coworkers (*12*), Klimmer (*8*), Jensen (*7*), Smith and Little (*11*), Birch and Gilman (*2*), Hadley (*4*), Huddleson (*6*), Fitch and Boyd (*3*), Hart and Traum (*5*), Lubbehusen (*9*), and others. The substances employed as immunizing agents have included vaccines, prepared from living abortion bacteria of different degrees of virulence, bacterins consisting of suspensions of the abortion microorganism killed by subjection to heat and chemicals, serum with a high agglutinating titer for *Bacterium abortus*, sero vaccines, and sero bacterins.

Vaccines consisting of viable abortion bacteria, unless of unusually low virulence, have commonly been administered to unbred animals only. Bacterins and serums have been used both before and after breeding.

In some of the earlier investigations, before it was well understood that the abortion microorganism frequently invades and establishes itself in the udders of cows, the immunologist was principally concerned in testing the efficacy of biological substances in preventing the occurrence of abortions. Later studies have not only given consideration to this feature but have frequently been so planned and pursued as to reveal detrimental features as well as beneficial effects resulting from the procedures.

Many workers in this field believe that the degree of resistance to *Bacterium abortus* that can be imparted to susceptible stock by the use of abortion bacterin and serum is too meager in amount and of too brief duration to render such products of practical value in dealing with this disease. The available experimental data seem to justify this belief. Vaccines consisting of viable abortion bacteria, on the other hand, have yielded results that have been a source of considerable encouragement to both the investigator and the stockowner. Experiments with selected stock have plainly and repeatedly demonstrated that the vaccination of unbred, susceptible heifers about two

[1] Received for publication June 27, 1930; issued November, 1930.

[2] The writer thanks H. E. Smith for helpful assistance in making pregnancy examinations and agglutination tests and in otherwise aiding in the vaccination experiment.

[3] Reference is made by number (italic) to Literature Cited, p. 689.

months before their breeding results in the development of an immunity that is both pronounced and lasting.

One of the arguments against vaccination has been that the administration of living suspensions of *Bacterium abortus* is followed by localization of the infection within the udder from which it may subsequently be eliminated for long periods of time. This characteristic has been noted with greater frequency after the vaccination of unbred cows with functioning udders than unbred heifers as they approach breeding age.

OBJECTS OF THE INVESTIGATION

The objects of the present investigation were twofold: (1) To determine whether a demonstrable and lasting degree of immunity could be engendered in bovines by their vaccination during calfhood rather than at their approach to or attainment of maturity and (2) to determine the ability of calves to resist the prolonged or permanent establishment of *Bacterium abortus* infection in their bodies following the subcutaneous administration of the microorganism.

The original plan was to select for vaccination calves 3 to 4 months of age, for in the event the experiment yielded encouraging results it seemed possible that the method might be an appropriate one to employ at calf-branding time in infected herds maintained under range or semirange conditions.

EXPERIMENTAL ANIMALS

Twenty calves were procured for this work during May and June, 1925. They were assembled from herds in the vicinity of Washington, D. C., by a cattle dealer and delivered at the Bureau of Animal Industry Experiment Station, Bethesda, Md. It was impracticable to obtain information of value with reference to infectious abortion in the herds from which they were obtained. Some difficulty was experienced in procuring calves of the desired age, and it finally became necessary to accept animals that had the appearance of being from five to eight months in development. The calves seemed to be reasonably well bred, probably the progeny of grade stock. Their weights ranged from 275 to 400 pounds.

TABLE 1.—*Results of the agglutination test for infectious abortion obtained from 20 calves purchased for vaccine experiment* [a]

Calf No.	Results when quantity of blood serum (cubic centimeters) indicated was used						Calf No.	Results when quantity of blood serum (cubic centimeters) indicated was used					
	0.04	0.02	0.01	0.005	0.002	0.001		0.04	0.02	0.01	0.005	0.002	0.001
1263	−	−	−	−	−	−	1275	−	−	−	−	−	−
1264	−	−	−	−	−	−	1276	−	−	−	−	−	−
1265	−	−	−	−	−	−	1277	−	−	−	−	−	−
1266	−	−	−	−	−	−	1278	−	−	−	−	−	−
1267	−	−	−	−	−	−	1279	+	P	−	−	−	−
1268	−	−	−	−	−	−	1280	−	−	−	−	−	−
1269	−	−	−	−	−	−	1281	−	−	−	−	−	−
1272	+	+	P	−	−	−	1302	−	−	−	−	−	−
1273	−	−	−	−	−	−	1303	−	−	−	−	−	−
1274	−	−	−	−	−	−	1304	−	−	−	−	−	−

[a] Key:
+ = Pronounced agglutination.
P = Partial agglutination.
− = No agglutination.

An agglutination test for infectious abortion made soon after the calves were received resulted in the data shown in Table 1. Calf 1272 reacted in a dilution of 1 to 100 and calf 1279 in a dilution of 1 to 50. These animals consequently were not utilized in the experiment. Of the remaining 18, 12 were used as principals and 6 as controls.

MATERIALS AND METHODS

It was not definitely known what degree of virulence *Bacterium abortus* strains used in the preparation of vaccine should possess in order to be capable, when administered subcutaneously to virgin animals, of conferring an appreciable degree of immunity without at the same time causing a permanent infection of the udders. Hence, it was proposed to give this feature consideration.

Three different lots of vaccine were accordingly prepared. *Bacterium abortus* strains 8, 10, and 11 that had been propagated on artificial culture media for approximately eight years were used in the preparation of the lot of vaccine administered to calves 1263, 1264, 1265, 1266, 1267, and 1302. Strain 19 that had been isolated one and one-half years previously was used in the preparation of the vaccine administered to calves 1268, 1276, and 1277, and *Bact. abortus* strain 88, which was the third transfer of this isolation, was used in the preparation of the vaccine administered to calves 1273, 1274, and 1278.

Slants of 3 per cent glycerin agar in 18-mm. tubes were sown with the different *Bacterium abortus* strains three days before the vaccination of the animals. The suspensions were prepared immediately preceding their use. The 20 c. c. dose which each of the 12 principals received corresponded to the quantity of growth propagated on one tube of medium and represented in density a Gates nephelometer reading of about 1 cm. The vaccine was introduced subcutaneously at four different points on the sides of the neck and thorax in an endeavor to prevent severe swelling or abscess formation. Although no discharging abscesses developed at the points of inoculation it seems possible that the dose should have been reduced, for somewhat pronounced swellings appeared, where the injections were made, that persisted for several days. On the second and third days after the injections, moreover, the calves were sluggish and refused feed, and with one exception exhibited much elevated temperatures. Calf 1273, although showing no increase in temperature and but average local reactions, appeared to be the most seriously affected of the group. She refused feed for a week and was not inclined to mingle with the other calves. During this period she appeared to dislike to stand or to move about.

The temperatures of the animals before vaccination and for several days following are shown in Table 2. The somewhat elevated preinjection temperatures probably resulted partly from the excitement induced by catching and confining and partly from the high atmospheric temperature which prevailed at the time.

The vaccinated and control animals were kept in separate fields for about three months, when they were placed in the same stable and thereafter were run together. At monthly intervals blood samples were obtained and subjected to the agglutination test.

TABLE 2.—*Temperatures of calves immediately preceding vaccination, September 15, 1925, and on five subsequent dates*

Calf No.	Preinjection temperature on Sept. 15, 1925	Postinjection temperature on—				
		Sept. 17, 1925	Sept. 18, 1925	Sept. 19, 1925	Sept. 21, 1925	Sept. 23, 1925
	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*
1263	105.2	108.4	108.0	105.4	105.5	103.8
1264	104.0	107.2	107.2	104.8	104.1	101.4
1265	103.8	106.6	106.2	105.6	105.0	102.4
1266	104.0	107.8	106.8	104.8	105.0	102.4
1267	104.0	107.2	106.8	104.2	103.5	102.6
1268	103.0	107.2	106.9	103.8	104.1	102.5
1273	103.8	103.4	102.9	102.0	103.2	101.5
1274	103.6	106.8	106.0	104.8	105.4	103.0
1276	104.4	107.8	108.0	106.4	106.4	102.6
1277	104.6	107.4	107.0	105.4	106.5	102.0
1278	103.4	106.8	106.8	104.2	104.8	102.4
1302	106.0	108.2	107.6	106.6	106.4	104.8

When blood samples were being taken on December 10, 1925, approximately three months after the vaccination date, it was observed that the udder of heifer 1273, one of the animals which received the most recently isolated strain of *Bacterium abortus*, showed some distention. She was consequently isolated and milk serum obtained from her udder. The establishment of *Bact. abortus* infection in this animal seemed to be indicated by the fact that her blood-serum reactions had shown little tendency to recede in intensity. Failure was experienced, however, in demonstrating the presence of abortion bacteria in her udder secretions at this time, through guinea-pig-inoculation tests. A month later she was again placed with the remainder of the group.

Breeding of the heifers was begun during the middle of June, 1926. A vigorous 2-year-old bull that gave negative results to the agglutination test for infectious abortion was used for the entire group. Before all the animals had conceived one of the controls, No. 1281, became badly injured and, in consequence, had to be killed. One of the vaccinated animals, No. 1302, was found dead about a month after vaccination. An autopsy indicated that death was induced by internal hemorrhage, there being a rupture of the posterior vena cava a few inches from the heart. The rupture was associated with embolus formation. An effort was made to isolate *Bacterium abortus* from the embolus and the diseased walls of the vein as well as from other organs, but without success. There now remained in the experiment 16 heifers, 11 of which were principals and 5 controls.

On November 23, 1926, a fetus from a *Bacterium abortus* infected herd was available as exposure material for this group of animals, in all of which pregnancy had now been definitely diagnosed.

After small amounts of the stomach and intestinal contents had been sown on serum agar to determine the presence of *Bact. abortus* infection, the stomach content was drained into a flask. The intestinal content was washed out with saline. These materials were stored at refrigerator temperature. When the presence of *Bact. abortus* in large numbers was definitely established in these materials, they were strained through cheesecloth to remove the larger particles and made up to a 2,500 c. c. amount with physiological saline solution.

On November 26, 1926, each of the 16 pregnant heifers received 150 c. c. of the material by way of mouth.

A second fetus from another infected herd was available November 26, 1926. This fetus was handled in a manner similar to the one previously described. After the presence of *Bacterium abortus* in its digestive contents was established, 2,100 c. c. of a suspension of stomach and intestinal contents was used for a second exposure of the 16 heifers November 29, 1926. Each heifer at this time received 125 c. c. of the material by way of mouth.

When calving or aborting occurred, guinea pigs were inoculated with colostrum. Guinea pigs were also injected with placental emulsions or uterine exudate, unless it seemed evident that cultural results with aborted fetuses would reveal the presence of *Bacterium abortus* infection in the uteri of the aborting animals.

RESULTS OF FIRST PREGNANCIES

During their first pregnancies the 11 vaccinated animals produced 11 living calves. These, with one exception, continued to thrive for a year or longer, when they were utilized for other experimental purposes. The exception was the calf of heifer 1276, which died nine days after birth, and at autopsy showed severe inflammation of the intestines. A bipolar staining organism was isolated from the blood of this calf which may have caused its death. There was little evidence that its illness was in any way associated with *Bacterium abortus* infection.

Of the five controls, three, Nos. 1269, 1303, and 1304, aborted. *Bacterium abortus* was isolated from their fetuses by cultural methods and from samples of their colostrum through guinea-pig inoculations. Two of the controls, Nos. 1275 and 1280, produced vigorous calves, although heifer 1275, 47 days after calving, developed an agglutination reaction to *Bact. abortus* infection of a titer of 1 to 200, which was maintained for about one month. Efforts to isolate the abortion organism from her milk slightly subsequent to this period was attended with failure.

These two animals, Nos. 1275 and 1280, may have possessed unusual resistance to the infection, a supposition somewhat strengthened by the fact that on receiving large doses of *Bacterium abortus* suspensions at a later date when again pregnant, intravenously and through the teat canals, they produced, at the expiration of normal periods of pregnancy, living vigorous calves. Before the original intentional exposure both of these heifers had given suspicious reactions to the agglutination test on different occasions, a feature that may have accounted in a measure for their apparent resistance.

Although 11 living calves were produced by the 11 vaccinated animals during their first pregnancies the results were slightly less encouraging than this performance would imply. Although the agglutination reaction of heifer 1263 had returned to negative four months after vaccination and continued to remain negative or practically so for 15 months, it thereupon became markedly positive and remained thus for many months. The immunity which the vaccine afforded in her case was seemingly insufficient to enable her to resist the degree of *Bacterium abortus* exposure to which she was subsequently subjected. Although her calf was carried nearly the normal

period of gestation and seemed to exhibit normal vigor, the presence of *Bact. abortus* was demonstrated both in her placenta and colostrum.

Heifer 1273, in which *Bacterium abortus* seemed to have been established by the vaccine, although she produced a seemingly normal calf at the expiration of a 279-day gestation period, nevertheless eliminated the infection in her colostrum, as was demonstrated by guinea-pig inoculation results. Guinea pigs which were injected with placental emulsions, however, failed to acquire the disease.

Heifer 1263, as previously mentioned, received vaccine prepared from *Bacterium abortus* strains 8, 10, and 11 that had been propagated for about eight years on artificial culture media, whereas heifer 1273 received vaccine prepared from *Bact. abortus* strain 88, which was the third transfer of this strain. The results obtained with heifers 1263 and 1273 were, therefore, such as might possibly be anticipated in some cases when strains representing extremes in virulence are used for vaccine preparation.

TABLE 3.—*Immunization results in animals vaccinated during calfhood and later subjected to artificial exposure to Bacterium-abortus-infected materials*

Animal No.	Date of vaccinations	Date of breeding	Date of exposure	Gestation period	Outcome of pregnancy	Results of uterine examinations for *Bacterium abortus*	Results of colostrum examinations for *Bacterium abortus*
TREATED WITH ABORTION VACCINE							
	1925	1926	1926	*Days*			
1263	Sept. 15	Aug. 31 Oct. 11	Nov. 26 Nov. 29	277	Living calf	Positive	Positive.
1264	do	June 22	Nov. 26 Nov. 29	284	do	Negative	Negative.
1265	do	Aug. 27	Nov. 26 Nov. 29	279	do	do	Do.
1266	do	June 18	Nov. 26 Nov. 29	275	do	do	Do.
1267	do	June 21	Nov. 25 Nov. 29	268	do	do	Do.
1268	do	July 8	Nov. 25 Nov. 29	282	do	do	Do.
1273	do	June 24	Nov. 26 Nov. 29	279	do	do	Positive.
1274	do	[a]	Nov. 26 Nov. 29		do	do	Negative.
1276	do	July 1	Nov. 26 Nov. 29	277	do	do	Do.
1277	do	July 23	Nov. 25 Nov. 29	282	do	do	Do.
1278	do	June 23	Nov. 26 Nov. 29	238	do	do	Do.
CONTROLS							
1269	[b]	June 20 July 8 Oct. 23	Nov. 26 Nov. 29	173	Abortion	Positive	Positive.
1275	[b]	June 20	Nov. 26 Nov. 29	279	Living calf	Negative	Negative.
1280	[b]	June 22	Nov. 26 Nov. 29	283	do	do	Do.
1303	[b]	July 3	Nov. 26 Nov. 29	215	Abortion	Positive	Positive.
1304	[b]	June 27	Nov. 25 Nov. 29	236	do	do	Do.

[a] No record.

[b] Not vaccinated.

Table 3 shows the date of vaccination, dates of breeding and of *Bacterium abortus* exposures, length of gestation periods, outcome of pregnancies, and results of uterine and colostrum examinations.

RESULTS OF SECOND PREGNANCIES

During the spring of 1928 the 11 vaccinated animals, without further treatment, were again bred to a bull giving negative results to the abortion test. The purpose was to gain some information as to the permanence of immunity engendered by calfhood vaccination. While it was realized that the ingestion exposure previously practiced complicated the problem of determining the lasting immunizing effects of vaccination alone, it was nevertheless believed that the existing conditions of exposure might not differ materially from the conditions encountered by vaccinated calves when in contact with infected herds.

Pregnancy examinations made during July and August, 1928, indicated that all 11 vaccinated heifers had conceived. Pregnancy was also determined in five other animals giving negative results to the agglutination test for the disease, and these animals were used as controls during the second pregnancies of the vaccinated group. This control group previously had been mated to an abortion-disease-free bull as indicated by the agglutination test. One of the controls, No. 1360, was a cow, the other four, Nos. 1419, 1425, 1426, and 1453, were heifers.

The *Bacterium abortus* exposure material used in this second period of the experiment was similar to that employed during the preceding pregnancies of the vaccinated animals. Two aborted fetuses were obtained from different herds on July 31 and August 6, 1928. Cultural work with these fetuses indicated that they were heavily infected with the abortion microorganism. The intestines were cut into small pieces with scissors and immersed and agitated in saline. The suspension was then passed through a wire sieve to remove the intestinal musculature. The suspension of intestinal contents was made up in each case to about 2,500 c. c. It was stored at a temperature of about 40° F. for several days, until cultural results were ascertained, before being used. To every experimental cow on each date of exposure was administered by way of mouth 100 c. c. of the material. Six days intervened between the times the cows were drenched, the first on August 3, 1928, the second on August 9. On the dates of drenching, subcutaneous inoculations of the exposure materials were made, into guinea pigs, to establish the infectiousness of the materials. These inoculations resulted in the development of marked lesions of abortion disease.

The results obtained after the second conception of the vaccinated animals did not differ markedly from those of the first. The 11 vaccinated animals produced 10 living calves, all of which made remarkably good growth.

Cow 1273 aborted at the expiration of a 176-day gestation period. *Bacterium abortus* could not be established as a causative factor, however, even though cultural examinations of the fetus were supplemented by guinea-pig inoculations of placental emulsions, colostrum, and emulsions of fetal stomach and intestinal contents. *Bacillus coli* was the predominating type of infection isolated from the

placenta. Cow 1263, from whose placenta and colostrum *Bact. abortus* was isolated at the previous pregnancy, although yielding positive colostrum results at this time, did not show *Bact. abortus* infection in the uterus, since guinea pigs inoculated with placental emulsions failed to acquire abortion disease. The uterine and colostrum results of the remaining nine vaccinated animals, as determined by guinea-pig inoculations at times of parturition, were negative.

Of the five control animals one heifer, No. 1426, aborted 218 days after her last breeding date. *Bacterium abortus* was isolated from her fetus by cultural means and from her colostrum by guinea-pig inoculations. Heifer 1425 expelled a weak calf on the two hundred fifty-eighth day of gestation. From the placenta and colostrum of this animal *Bact. abortus* was isolated. Heifer 1419 was in labor on the two hundred sixty-ninth day of gestation and required assistance in the removal of her calf, which was dead. A search for *Bact. abortus*, both culturally and through guinea-pig inoculations of placental and fetal materials, gave negative results. The serological reactions of this heifer supported the cultural and inoculation results.

Two of the five control animals produced vigorous calves.

TABLE 4.—*Immunization results during second pregnancies of animals vaccinated during calfhood when again subjected to Bacterium-abortus-ingestion exposure*

Animal No.	Date of breeding	Date of exposure	Gestation period	Outcome of pregnancy	Results of uterine examinations for *Bacterium abortus*	Results of colostrum examinations for *Bacterium abortus*
	1928	1928	*Days*			
TREATED BEFORE FIRST PREGNANCY WITH ABORTION VACCINE						
1263	Apr. 11	Aug. 3 to Aug. 9	275	Living calf	Negative	Positive.
1264	Mar 27	Aug. 3 to Aug. 9	286	do	do	Negative.
1265	Apr. 9	Aug. 3 to Aug. 9	286	do	do	Do.
1266	Apr. 4	Aug. 3 to Aug. 9	261	do	do	Do.
1267	Mar. 24	Aug. 3 to Aug. 9	279	do	do	Do.
1268	Mar. 26	Aug. 3 to Aug. 9	280	do	do	Do.
1273	Mar. 21 / May 1	Aug. 3 to Aug. 9	176	Abortion	do	Do.
1274	Mar. 27	Aug. 3 to Aug. 9	282	Living calf	do	Do.
1276	Mar. 23	Aug. 3 to Aug. 9	281	do	do	Do.
1277	Mar. 22 / Apr. 21	Aug. 3 to Aug. 9	280	do	do	Do.
1278	Apr. 2	Aug. 3 to Aug. 9	276	do	do	Do.
CONTROLS						
1360	Feb. 6	Aug. 3 to Aug. 9	288	Living calf	Negative	Negative.
1419	Apr. 3	Aug. 3 to Aug. 9	269	Dead calf	do	Do.
1425	Apr. 4	Aug. 3 to Aug. 9	258	Living calf	Positive	Positive.
1426	Mar. 24 / Apr.[a] 14	Aug. 3 to Aug. 9	218	Abortion	do	Do.
1453	(a)	Aug. 3 to Aug. 9	.....	Living calf	Negative	Negative.

[a] No record.

Table 4 shows in condensed form the procedure followed and the results of the second phase of the calfhood-vaccination experiment.

Table 5 contains more complete records of the individual animals used in the experiment. In it are recorded the agglutination results that were obtained at varying intervals of time, the date of vaccination, dates of breeding, *Bacterium abortus* exposures, calvings, cultural determinations, and results of guinea-pig inoculations with colostrum, milk, and uterine products.

Those guinea pigs which were inoculated with the different materials and did not succumb to pneumonia, gastritis, or enteritis were killed in from five to eight weeks after inoculation, examined for the presence of lesions, and their blood subjected to the agglutination test for abortion disease. In some instances the spleens of the guinea pigs were cultured. This procedure was regarded as furnishing rather definite information as to the presence or absence of the infection in the inoculated pigs. The records of those guinea pigs that died soon after the injection, and that were not cultured or from which blood samples were not obtained for diagnostic determinations, are rarely entered in the table, because of the unreliability of the evidence which they furnished.

TABLE 5.—*Records of individual experiment animals* [a]

VACCINATED HEIFER 1263

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used [b]									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact. abortus* strains 8, 10, and 11).
Oct. 4, 1925	+	+	+	+	+	+	+	P	−	
Nov. 10, 1925	+	+	+	+	−	−				
Dec. 10, 1925	+	+	+	−	−	−				
Jan. 15, 1926	−	−	−	−	−	−				
Feb. 15, 1926	+	−	−	−	−	−				
Mar. 16, 1926	S	−	−	−	−	−				
Apr. 22, 1926	+	−	−	−	−	−				
May 21, 1926	−	−	−	−	−	−				
June 14, 1926	P	−	−	−	−	−				
July 20, 1926	−	−	−	−	−	−				
Aug. 24, 1926	−	−	−	−	−	−				Aug. 31, 1926, bred.
Sept. 22, 1926	−	−	−	−	−	−				Oct. 11, 1926, bred.
Nov. 23, 1926	−	−	−	−	−	−				Nov. 26, 1926, received [c] 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	−	−	−	−	−	−				
Jan. 6, 1927	−	−	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	−	−	−	−	−	−				
Feb. 4, 1927	−	−	−	−	−	−				
Mar. 7, 1927	−	−	−	−	−	−				
Mar. 21, 1927	−	−	−	−	−	−				
Apr. 4, 1927	−	−	−	−	−	−				
Apr. 19, 1927	+	+	+	+	P	−				
May 2, 1927	+	+	+	+	+	P				
May 17, 1927	+	+	+	+	+	+				
June 1, 1927	+	+	+	+	+	+	+	+	+	
June 16, 1927	+	+	+	+	+	+	+	+	+	
July 19, 1927	+	+	+	+	+	+	+	+	+	July 15, 1927, expelled a living calf (277 days); guinea pigs inoculated with emulsion of placenta and colostrum acquired abortion disease.
Aug. 6, 1927	+	+	+	+	+	+	+	+	+	
Sept. 21, 1927	+	+	+	+	+	+	−	−	−	

[a] Explanation of symbols: The plus sign indicates pronounced clumping of bacteria; P, partial clumping; S, slight trace of agglutination; minus sign (−), no evidence of clumping.

[b] Figures at head of columns indicate cubic centimeters of blood serum. They represent approximate dilutions of 1 to 25, 1 to 50, 1 to 100, 1 to 200, 1 to 500, 1 to 1,000, 1 to 2,000, 1 to 5,000, and 1 to 10,000, respectively, with the amount of antigen (1 c. c.) used.

[c] All exposures were made orally.

TABLE 5.—*Records of individual experiment animals*—Continued

VACCINATED HEIFER 1263—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
Oct. 20, 1927	+	+	+	+	+	−				Oct. 20, 1927, two guinea pigs inoculated with milk failed to acquire abortion disease.
Nov. 19, 1927	+	+	+	+	+	P	−			
Jan. 25, 1928	+	+	+	+	+	+	+	P	P	Jan. 25, 1928, three guinea pigs inoculated with milk acquired abortion disease.
June 6, 1928	+	+	+	+	+	P				
July 18, 1928	+	+	+	+	+	+				Apr. 11, 1928, bred.
Aug. 24, 1928	+	+	+	+	+	P				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	P	P	+	+	+	+				
Oct. 12, 1928	+	+	+	+	+	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	+	+	+	+	+	P				
Nov. 16, 1928	+	+	+	+	P	P				
Dec. 6, 1928	+	+	+	+	+	+				
Dec. 19, 1928	+	+	+	+	+	+				
Jan. 8, 1929	+	+	+	+	+	+				
Jan. 28, 1929	+	+	+	+	+	+				Jan. 11, 1929, expelled a living calf (275 days); 5 guinea pigs inoculated with emulsion of placenta failed to acquire abortion disease; 5 inoculated with colostrum contracted abortion disease.
Feb. 28, 1929	+	+	+	+	+	+				
Apr. 11, 1929	+	+	+	+	P	−				

VACCINATED HEIFER 1264

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact. abortus* strains 8, 10, and 11).
Oct. 4, 1925	+	+	+	+	+	+	+	8	−	
Nov. 10, 1925	+	+	+	+	−	−				
Dec. 10, 1925	+	+	+	8	−	−				
Jan. 15, 1926	P	−	−	−	−	−				
Feb. 15, 1926	+	−	−	−	−	−				
Mar. 16, 1926	+	8	−	−	−	−				
Apr. 22, 1926	+	−	−	−	−	−				
May 21, 1926	+	−	−	−	−	−				
June 14, 1926	+	−	−	−	−	−				June 22, 1926, bred.
July 20, 1926	P	−	−	−	−	−				
Aug. 24, 1926	P	−	−	−	−	−				
Sept. 22, 1926	P	−	−	−	−	−				
Nov. 23, 1926	P	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	8	−	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 6, 1927	P	−	−	−	−	−				
Jan. 22, 1927	8	−	−	−	−	−				
Feb. 4, 1927	P	−	−	−	−	−				
Mar. 7, 1927	P	−	−	−	−	−				
Mar. 21, 1927	−	−	−	−	−	−				
Apr. 4, 1927	−	−	−	−	−	−				Apr. 2, 1927, expelled a living calf (284 days); 4 guinea pigs inoculated with uterine material and 4 with colostrum failed to develop abortion disease.
Apr. 19, 1927	−	−	−	−	−	−				
May 2, 1927	−	−	−	−	−	−				
May 17, 1927	P	−	−	−	−	−				
June 16, 1927	−	−	−	−	−	−				June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to acquire abortion disease.
July 19, 1927	−	−	−	−	−	−				
Aug. 6, 1927	−	−	−	−	−	−				
Sept. 21, 1927	−	−	−	−	−	−				
Oct. 20, 1927	−	−	−	−	−	−				
Nov. 19, 1927	−	−	−	−	−	−				
Jan. 25, 1928	−	−	−	−	−	−				Mar. 27, 1928, bred.
June 6, 1928	−	−	−	−	−	−				
July 18, 1928	P	−	−	−	−	−				
Aug. 24, 1928	P	−	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	+	−	−	−	−	−				
Oct. 12, 1928	−	−	−	−	−	−				
Oct. 27, 1928	+	8	−	−	−	−				Aug 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Nov. 16, 1928	−	−	−	−	−	−				
Dec. 6, 1928	8	−	−	−	−	−				
Dec. 19, 1928	−	−	−	−	−	−				
Jan. 8, 1929	−	−	−	−	−	−				Jan. 7, 1929, expelled a living calf (286 days); 4 guinea pigs inoculated with an emulsion of placenta and 6 with colostrum failed to acquire abortion disease.
Jan. 28, 1929	−	−	−	−	−	−				

TABLE 5.—*Records of individual experiment animals*—Continued

VACCINATED HEIFER 1265

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				
Oct. 4, 1925	+	+	+	+	+	+	+	+	+	Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact. abortus* strains 8, 10, and 11).
Nov. 10, 1925	+	+	+	+	P	−				
Dec. 10, 1925	+	+	+	P	−	−				
Jan. 15, 1926	+	P	−	−	−	−				
Feb. 15, 1926	+	+	P	P	−	−				
Mar. 16, 1926	+	+	P	−	−	−				
Apr. 22, 1926	+	P	−	−	−	−				
May 21, 1926	+	+	P	−	−	−				
June 14, 1926	+	+	+	−	−	−				
July 20, 1926	+	+	−	−	−	−				
Aug. 24, 1926	+	+	−	−	−	−				Aug. 27, 1926, bred.
Sept. 22, 1926	+	S	−	−	−	−				
Nov. 23, 1926	+	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	P	−	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 6, 1927	+	P	−	−	−	−				
Jan. 22, 1927	+	+	−	−	−	−				
Feb. 4, 1927	+	P	−	−	−	−				
Mar. 7, 1927	+	+	−	−	−	−				
Mar. 21, 1927	+	−	−	−	−	−				
Apr. 4, 1927	+	P	−	−	−	−				
Apr. 19, 1927	+	+	−	−	−	−				
May 2, 1927	+	P	−	−	−	−				
May 17, 1927	+	+	−	−	−	−				
June 1, 1927	+	P	−	−	−	−				June 2, 1927, expelled a living calf (279 days); 4 guinea pigs inoculated with uterine material and 4 with colostrum failed to contract abortion disease.
June 16, 1927	+	−	−	−	−	−				
July 19, 1927	+	P	−	−	−	−				
Aug. 6, 1927	+	−	−	−	−	−				
Sept. 21, 1927	+	−	−	−	−	−				June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to acquire abortion disease.
Oct. 20, 1927	P	−	−	−	−	−				
Nov. 19, 1927	S	−	−	−	−	−				
Jan. 25, 1928	P	−	−	−	−	−				Apr. 9, 1928, bred.
June 6, 1928	+	−	−	−	−	−				
July 18, 1928	+	S	−	−	−	−				
Aug. 24, 1928	+	S	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	+	+	−	−	−	−				
Oct. 12, 1928	P	−	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	+	P	−	−	−	−				
Nov. 16, 1928	+	−	−	−	−	−				
Dec. 6, 1928	+	S	−	−	−	−				
Dec. 19, 1928	P	S	−	−	−	−				
Jan. 8, 1929	+	−	−	−	−	−				Jan. 20, 1929, expelled a living calf (286 days); 6 guinea pigs inoculated with placental emulsion and 6 with colostrum failed to acquire abortion disease.
Jan. 28, 1929	+	−	−	−	−	−				
Feb. 28, 1929	+	−	−	−	−	−				
Apr. 11, 1929	+	−	−	−	−	−				

VACCINATED HEIFER 1266

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				
Oct. 4, 1925	+	+	+	+	+	+	+	−	−	Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact. abortus* strains 8, 10, and 11).
Nov. 10, 1925	+	+	+	+	P	−				
Dec. 10, 1925	+	+	+	+	−	−				
Jan. 15, 1926	+	P	S	−	−	−				
Feb. 15, 1926	+	+	−	−	−	−				
Mar. 16, 1926	+	+	−	−	−	−				
Apr. 22, 1926	+	+	−	−	−	−				
May 21, 1926	+	P	−	−	−	−				
June 14, 1926	+	+	+	−	−	−				June 18, 1926, bred.
July 20, 1926	+	P	−	−	−	−				
Aug. 24, 1926	+	−	−	−	−	−				
Sept. 22, 1926	P	−	−	−	−	−				
Oct. 19, 1926	+	−	−	−	−	−				

TABLE 5.—*Records of individual experiment animals*—Continued

VACCINATED HEIFER 1266—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
Nov. 23, 1926	+	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	+	P	−	−	−	−				
Jan. 6, 1927	+	P	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	+	P	−	−	−	−				
Feb. 4, 1927	+	P	−	−	−	−				
Feb. 19, 1927	+	+	−	−	−	−				
Mar. 7, 1927	+	P	−	−	−	−				Mar. 20, 1927, expelled a living calf (275 days); 4 guinea pigs inoculated with uterine material and 4 with colostrum failed to acquire abortion disease.
Apr. 4, 1927	+	+	−	−	−	−				
Apr. 19, 1927	+	S	−	−	−	−				
May 2, 1927	+	−	−	−	−	−				
May 17, 1927	P	−	−	−	−	−				
June 1, 1927	+	−	−	−	−	−				June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to acquire abortion disease.
June 16, 1927	P	−	−	−	−	−				
July 19, 1927	+	+	−	−	−	−				
Aug. 6, 1927	+	+	−	−	−	−				
Sept. 21, 1927	−	−	−	−	−	−				
Oct. 20, 1927	−	−	−	−	−	−				
Nov. 19, 1927	−	−	−	−	−	−				
Jan. 25, 1928	+	−	−	−	−	−				Apr. 4, 1928, bred.
June 6, 1928	−	−	−	−	−	−				
July 18, 1928	+	S	−	−	−	−				
Aug. 24, 1928	+	P	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	+	+	−	−	−	−				
Oct. 12, 1928	P	−	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	+	−	−	−	−	−				
Nov. 16, 1928	+	P	−	−	−	−				
Dec. 6, 1928	−	−	−	−	−	−				
Dec. 19, 1928	+	−	−	−	−	−				Dec. 21, 1928, expelled a living calf (261 days); 6 guinea pigs inoculated with an emulsion of the placenta and 6 with colostrum failed to acquire abortion disease; streptococcic infection of the placenta.
Jan. 8, 1929	P	−	−	−	−	−				
Jan. 28, 1929	P	−	−	−	−	−				
Feb. 28, 1929	P	−	−	−	−	−				
Apr. 11, 1929	P	−	−	−	−	−				

VACCINATED HEIFER 1267

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	S	−	−	−	−	−				
Oct. 4, 1925	+	+	+	+	+	+	+	S	−	Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact. abortus* strains 8, 10, and 11).
Nov. 10, 1925	+	+	+	P	−	−				
Dec. 10, 1925	+	+	P	−	−	−				
Jan. 5, 1926	+	P	−	−	−	−				
Feb. 15, 1926	+	P	−	−	−	−				
Mar. 16, 1926	+	−	−	−	−	−				
Apr. 22, 1926	+	−	−	−	−	−				
May 21, 1926	+	−	−	−	−	−				
June 14, 1926	+	−	−	−	−	−				June 21, 1926, bred.
July 20, 1926	+	P	−	−	−	−				
Aug. 24, 1926	+	S	−	−	−	−				
Sept. 22, 1926	P	S	−	−	−	−				
Oct. 19, 1926	+	S	−	−	−	−				
Nov. 23, 1926	P	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	+	−	−	−	−	−				
Jan. 6, 1927	P	−	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	P	S	−	−	−	−				
Feb. 4, 1927	+	P	−	−	−	−				
Feb. 19, 1927	S	S	−	−	−	−				
Mar. 7, 1927	+	−	−	−	−	−				
Mar. 21, 1927	+	−	−	−	−	−				Mar. 16, 1927, expelled a living calf (268 days); 4 guinea pigs inoculated with uterine material and 4 with colostrum failed to contract abortion disease.
Apr. 4, 1927	P	−	−	−	−	−				
Apr. 19, 1927	−	−	−	−	−	−				
May 2, 1927	−	−	−	−	−	−				
May 17, 1927	−	−	−	−	−	−				

TABLE 5.—*Records of individual experiment animals*—Continued

VACCINATED HEIFER 1267—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
June 1, 1927	+	P	−	−	−	−				June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to acquire abortion disease.
June 16, 1927	−	−	−	−	−	−				
July 19, 1927	+	P	−	−	−	−				
Aug. 6, 1927	+	+	−	−	−	−				
Sept. 21, 1927	−	−	−	−	−	−				
Oct. 20, 1927	−	−	−	−	−	−				Oct. 20, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to acquire abortion disease.
Nov. 19, 1927	−	−	−	−	−	−				
Jan. 25, 1928	+	−	−	−	−	−				Mar. 24, 1928, bred.
June 6, 1928	+	−	−	−	−	−				
July 18, 1928	+	S	−	−	−	−				
Aug. 24, 1928	+	−	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	+	+	−	−	−	−				
Oct. 12, 1928	−	−	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	+	P	−	−	−	−				
Nov. 16, 1928	+	P	−	−	−	−				
Dec. 6, 1928	P	−	−	−	−	−				
Dec. 19, 1928	+	−	−	−	−	−				Dec. 28, 1928, expelled a living calf (279 days); 6 guinea pigs inoculated with uterine material and 6 with colostrum failed to acquire abortion disease.
Jan. 8, 1929	+	−	−	−	−	−				
Jan. 28, 1929	P	−	−	−	−	−				
Feb. 28, 1929	P	−	−	−	−	−				
Apr. 11, 1929	+	−	−	−	−	−				

VACCINATED HEIFER 1268

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact. abortus* strain 19).
Oct. 4, 1925	+	+	+	+	+	+	+	+	−	
Nov. 10, 1925	+	+	+	+	+	+	+	S	−	
Dec. 10, 1925	+	+	+	+	S	−				
Jan. 15, 1926	+	+	+	−	−	−				
Feb. 15, 1926	+	+	P	−	−	−				
Mar. 16, 1926	+	+	P	−	−	−				
Apr. 22, 1926	+	+	+	−	−	−				
May 21, 1926	+	+	+	−	−	−				
June 14, 1926	+	+	−	−	−	−				
July 20, 1926	+	+	−	−	−	−				July 8, 1926, bred.
Aug. 24, 1926	+	P	−	−	−	−				
Sept. 22, 1926	+	P	−	−	−	−				
Oct. 19, 1926	+	+	−	−	−	−				
Nov. 23, 1926	+	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	+	P	−	−	−	−				
Jan. 6, 1927	P	P	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	P	P	−	−	−	−				
Feb. 4, 1927	+	+	−	−	−	−				
Feb. 19, 1927	+	+	−	−	−	−				
Mar. 7, 1927	+	P	−	−	−	−				
Mar. 21, 1927	+	+	−	−	−	−				
Apr. 4, 1927	+	+	−	−	−	−				
Apr. 19, 1927	+	+	−	−	−	−				
May 2, 1927	+	P	−	−	−	−				Apr. 16, 1927, expelled a living calf (282 days); 4 guinea pigs inoculated with colostrum failed to acquire abortion disease; 4 inoculated with uterine material died within 48 hours of septic infection.
May 17, 1927	+	P	−	−	−	−				
June 1, 1927	+	−	−	−	−	−				
June 16, 1927	+	P	−	−	−	−				
July 19, 1927	+	+	−	−	−	−				June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to contract abortion disease.
Aug. 6, 1927	+	+	−	−	−	−				
Sept. 21, 1927	+	−	−	−	−	−				
Oct. 20, 1927	+	−	−	−	−	−				
Nov. 19, 1927	+	−	−	−	−	−				
Jan. 25, 1928	+	P	−	−	−	−				Mar. 26, 1928, bred.
June 6, 1928	+	−	−	−	−	−				
July 18, 1928	+	P	−	−	−	−				
Aug. 24, 1928	+	−	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	+	P	−	−	−	−				

TABLE 5.—*Records of individual experiment animals*—Continued

VACCINATED HEIFER 1268—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
Oct. 12, 1928	+	−	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	+	P	−	−	−	−				
Nov. 16, 1928	+	S	−	−	−	−				
Dec. 6, 1928	S	−	−	−	−	−				
Dec. 19, 1928	P	−	−	−	−	−				Dec. 31, 1928, expelled a living calf (280 days); 6 guinea pigs inoculated with uterine material and 6 with colostrum failed to contract abortion disease.
Jan. 8, 1929	+	P	−	−	−	−				
Jan. 28, 1929	+	−	−	−	−	−				
Feb. 28, 1929	+	P	−	−	−	−				
Apr. 11, 1929	+	P	−	−	−	−				

VACCINATED HEIFER 1273

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact. abortus* strain 88).
Oct. 4, 1925	+	+	+	+	+	+	+	−	−	
Nov. 10, 1925	+	+	+	+	+	+	S	−	−	
Dec. 10, 1925	+	+	+	+	+	+	+	+	+	
Jan. 15, 1926	+	+	+	+	+	+				
Feb. 15, 1926	+	+	+	+	+	+				
Mar. 16, 1926	+	+	+	+	+	+				
Apr. 22, 1926	+	+	+	+	+	+				
May 21, 1926	+	+	+	+	+	+				
June 14, 1926	+	+	+	+	+	+				June 24, 1926, bred.
July 20, 1926	+	+	+	+	P	−				
Aug. 24, 1926	+	+	+	+	−	−				
Sept. 22, 1926	+	+	+	S	−	−				
Oct. 19, 1926	+	+	+	P	−	−				
Nov. 23, 1926	+	+	+	+	+	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	+	+	+	+	−	−				
Jan. 6, 1927	+	+	+	+	+	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	+	+	+	+	+	P				
Feb. 4, 1927	+	+	+	+	+	+				
Feb. 19, 1927	+	+	+	+	+	+				
Mar. 7, 1927	+	+	+	+	+	P				
Mar. 21, 1927	+	+	+	+	+	+				Mar. 30, 1927, expelled a living calf (279 days); 4 guinea pigs inoculated with uterine material failed to contract abortion disease; 4 inoculated with colostrum acquired abortion disease.
Apr. 4, 1927	+	+	+	+	+	−				
Apr. 19, 1927	+	+	+	+	P	−				
May 2, 1927	+	+	+	+	+	−				
May 17, 1927	+	+	+	+	+	−				May 17, 1927, 3 guinea pigs inoculated with a composite sample of milk failed to contract abortion disease.
June 1, 1927	+	+	+	+	P	−				
June 16, 1927	+	+	+	+	−	−				
July 19, 1927	+	+	+	+	+	−				
Aug. 6, 1927	+	+	+	+	−	−				
Sept. 21, 1927	+	+	S	S	−	−				
Oct. 20, 1927	+	+	+	−	−	−				Oct. 20, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to acquire abortion disease.
Nov. 19, 1927	+	+	+	P	−	−				
Jan. 25, 1928	+	+	+	+	−	−				Jan. 25, 1928, 3 guinea pigs inoculated with a composite sample of milk failed to contract abortion disease.
June 6, 1928	+	+	P	−	−	−				
July 18, 1928	+	+	+	P	−	−				Mar. 21, 1928, bred.
Aug. 24, 1928	+	+	+	−	−	−				May 1, 1928, bred.
Sept. 19, 1928	+	+	+	+	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 12, 1928	+	+	+	−	−	−				
Oct. 27, 1928	+	+	+	P	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Nov. 16, 1928	+	+	+	P	−	−				
Dec. 6, 1928	+	+	+	P	−	−				Oct. 24, 1928, aborted (176 days); cultural results with fetus negative for *Bact. abortus;* 4 guinea pigs inoculated with placental emulsion, 4 with stomach and intestinal contents of fetus, and 6 with colostrum failed to contract abortion disease.
Dec. 19, 1928	+	+	+	+	−	−				
Jan. 8, 1929	+	+	+	P	−	−				
Jan. 28, 1929	+	+	+	P	−	−				
Feb. 28, 1929	+	+	+	+	−	−				
Apr. 11, 1929	+	+	+	+	−	−				

TABLE 5.—*Records of individual experiment animals*—Continued

VACCINATED HEIFER 1274

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				
Oct. 4, 1925	+	+	+	+	+	+	−			Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact. abortus* strain 88).
Nov. 10, 1925	+	+	+	+	−	−				
Dec. 10, 1925	+	+	−	−	−	−				
Jan. 15, 1926	P	S	−	−	−	−				
Feb. 15, 1926	P	−	−	−	−	−				
Mar. 16, 1926	+	+	−	−	−	−				
Apr. 22, 1926	−	−	−	−	−	−				
May 21, 1926	−	−	−	−	−	−				
June 14, 1926	+	−	−	−	−	−				
July 20, 1926	P	−	−	−	−	−				No breeding record.
Aug. 24, 1926	−	−	−	−	−	−				
Sept. 22, 1926	S	−	−	−	−	−				
Oct. 19, 1926	−	−	−	−	−	−				
Nov. 23, 1926	−	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	−	−	−	−	−	−				
Jan. 6, 1927	−	−	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	−	−	−	−	−	−				
Feb. 4, 1927	−	−	−	−	−	−				
Feb. 19, 1927	−	−	−	−	−	−				
Mar. 7, 1927	−	−	−	−	−	−				
Mar. 21, 1927	−	−	−	−	−	−				
Apr. 4, 1927	−	−	−	−	−	−				
Apr. 19, 1927	−	−	−	−	−	−				
May 2, 1927	−	−	−	−	−	−				
May 17, 1927	−	−	−	−	−	−				
June 1, 1927	−	−	−	−	−	−				
June 16, 1927	−	−	−	−	−	−				
July 19, 1927	−	−	−	−	−	−				
Aug. 6, 1927	−	−	−	−	−	−				
Sept. 21, 1927	−	−	−	−	−	−				Aug. 18, 1927, expelled a living calf; 2 guinea pigs inoculated with placental emulsion and 2 with colostrum failed to contract abortion disease.
Oct. 20, 1927	−	−	−	−	−	−				
Nov. 19, 1927	−	−	−	−	−	−				
Jan. 25, 1928	−	−	−	−	−	−				Mar. 27, 1928, bred.
June 6, 1928	−	−	−	−	−	−				
July 18, 1928	−	−	−	−	−	−				
Aug. 24, 1928	−	−	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	−	−	−	−	−	−				
Oct. 12, 1928	−	−	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	−	−	−	−	−	−				
Nov. 16, 1928	−	−	−	−	−	−				
Dec. 6, 1928	−	−	−	−	−	−				
Dec. 19, 1928	−	−	−	−	−	−				
Jan. 8, 1929	−	−	−	−	−	−				Jan. 3, 1929, expelled a living calf (282 days); 6 guinea pigs inoculated with placental emulsion and 6 with colostrum failed to contract abortion disease.
Jan. 28, 1929	−	−	−	−	−	−				
Feb. 28, 1929	−	−	−	−	−	−				
Apr. 11, 1929	−	−	−	−	−	−				

VACCINATED HEIFER 1276

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				
Oct. 4, 1925	+	+	+	+	+	+	+	+	S	Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact. abortus* strain 19).
Nov. 10 1925	+	+	+	+	+	S	−			
Dec. 10, 1925	+	+	+	+	P	−				
Jan. 15, 1926	+	+	+	+	−	−				
Feb. 15, 1926	+	+	S	−	−	−				
Mar. 16, 1926	+	+	S	−	−	−				
Apr. 22, 1926	+	+	P	−	−	−				
May 21, 1926	+	P	S	−	−	−				
June 14, 1926	+	+	−	−	−	−				
July 20, 1926	+	P	−	−	−	−				
Aug. 24, 1926	+	+	−	−	−	−				

TABLE 5.—*Records of individual experiment animals*—Continued

VACCINATED HEIFER 1276—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
Sept. 22, 1926	+	+	−	−	−	−				July 1, 1926, bred.
Oct. 19, 1926	+	+	−	−	−	−				
Nov. 23, 1926	+	+	−	−	−	−				Nov. 23, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	+	+	P	−	−	−				
Jan. 6, 1927	+	+	P							Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22 1927	+	+	P	−	−	−				
Feb. 4, 1927	+	+	−	−	−	−				
Feb. 19, 1927	+	+	S	−	−	−				
Mar. 7, 1927	+	S	−	−	−	−				
Mar. 21, 1927	+	+	P	−	−	−				
Apr. 4, 1927	+	P	−	−	−	−				Apr 4, 1927, expelled a living calf (277 days); 4 guinea pigs inoculated with placental emulsion and 4 with colostrum failed to acquire abortion disease.
Apr. 19, 1927	+	+	−	−	−	−				
May 2 1927	+	P	−	−	−	−				
May 17, 1927	+	P	−	−	−	−				
June 1, 1927	+	P	−	−	−	−				
June 16, 1927	+	P	−	−	−	−				June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to acquire abortion disease.
July 19, 1927	+	+	−	−	−	−				
Aug. 6, 1927	+	+	P	−	−	−				
Sept. 21, 1927	+	−	−	−	−	−				
Oct. 20, 1927	P	−	−	−	−	−				
Nov. 19, 1927	P	−	−	−	−	−				
Jan. 25, 1928	+	−	−	−	−	−				Mar. 23, 1928, bred.
June 6, 1928	−	−	−	−	−	−				
July 18, 1928	+	P	−	−	−	−				
Aug. 24, 1928	+	−	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	+	P	−	−	−	−				
Oct. 12, 1928	+	−	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	P	S	−	−	−	−				
Nov. 16 1928	+	P	−	−	−	−				
Dec. 6, 1928	−	−	−	−	−	−				
Dec. 19, 1928	+	−	−	−	−	−				Dec. 29, 1928, expelled a living calf (281 days); 6 guinea pigs inoculated with placental emulsion and 6 with colostrum failed to contract abortion disease.
Jan. 8 1929	+	−	−	−	−	−				
Jan. 28, 1929	P	−	−	−	−	−				
Feb. 28 1929	+	−	−	−	−	−				
Apr. 11, 1929	+	−	−	−	−	−				

VACCINATED HEIFER 1277

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				
Oct. 4, 1925	+	+	+	+	+	+	+	+	P	Sept. 15, 1925, subcutaneous injection 20 c. c abortion vaccine (*Bact. abortus* strain 19).
Nov. 11. 1925	+	+	+	+	P	−				
Dec. 10, 1925	+	+	+	+	P	−				
Jan. 15, 1926	+	+	+	+	−	−				
Feb. 15, 1926	+	+	S	−	−	−				
Mar. 16, 1926	+	+	P	−	−	−				
Apr. 22, 1926	+	+	−	−	−	−				
May 21, 1926	+	+	S	−	−	−				
June 14, 1926	+	+	−	−	−	−				
July 20, 1926	+	+	−	−	−	−				July 23, 1926, bred.
Aug. 24, 1926	+	+	−	−	−	−				
Sept. 22, 1926	+	+	−	−	−	−				
Oct. 19, 1926	+	+	−	−	−	−				
Nov. 23, 1926	+	P	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	+	P	−	−	−	−				
Jan. 6, 1927	+	P	−	−	−	−				
Jan. 22, 1927	+	+	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Feb. 4, 1927	+	+	−	−	−	−				
Feb. 19, 1927	+	+	−	−	−	−				
Mar. 7, 1927	+	−	−	−	−	−				
Mar. 21, 1927	+	+	S	−	−	−				
Apr. 4, 1927	+	+	−	−	−	−				
Apr. 19, 1927	+	−	−	−	−	−				May 1, 1927, expelled a living calf (282 days); 4 guinea pigs inoculated with placental emulsion and 4 with colostrum failed to contract abortion disease.
May 2, 1927	+	P	−	−	−	−				
May 17, 1927	+	+	−	−	−	−				
June 1, 1927	+	−	−	−	−	−				
June 16, 1927	+	−	−	−	−	−				
July 19, 1927	+	−	−	−	−	−				June 15, 1927, 3 guinea pigs inoculated with milk failed to contract abortion disease.
Aug. 6, 1927	+	−	−	−	−	−				
Sept. 21, 1927	+	−	−	−	−	−				
Oct. 20, 1927	+	−	−	−	−	−				
Nov. 19, 1927	+	−	−	−	−	−				

TABLE 5.—*Records of individual experiment animals*—Continued

VACCINATED HEIFER 1277—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
Jan. 25, 1928	+	P	−	−	−	−				Mar. 22, 1928, bred.
June 6, 1928	+	S	−	−	−	−				Apr. 21, 1928, bred.
July 18, 1928	+	P	−	−	−	−				
Aug. 24, 1928	+	P	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	+	+	−	−	−	−				
Oct. 12, 1928	+	S	−	−	−	−				
Oct. 27, 1928	+	P	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Nov. 16, 1928	+	P	−	−	−	−				
Dec. 6, 1928	+	+	−	−	−	−				
Dec. 19, 1928	+	P	−	−	−	−				
Jan. 8, 1929	+	+	−	−	−	−				
Jan. 28, 1929	+	P	−	−	−	−				Jan. 26, 1928, expelled a living calf (280 days); 6 guinea pigs inoculated with placental emulsion and 6 with colostrum failed to contract abortion disease.
Feb. 28, 1929	+	P	−	−	−	−				
Apr. 11, 1929	+	S	−	−	−	−				

VACCINATED HEIFER 1278

May 21, 1925	−	−	−	−	−	−				
June 23, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				Sept. 15, 1925, subcutaneous injection 20 c. c. abortion vaccine (*Bact. abortus* strain 88).
Oct. 4, 1925	+	+	+	+	+	+	+	+	P	
Nov. 10, 1925	+	+	+	+	+	−				
Dec. 10, 1925	+	+	+	+	+	−				
Jan. 15, 1926	+	+	−	−	−	−				
Feb. 15, 1926	+	P	−	−	−	−				
Mar. 16, 1926	+	P	−	−	−	−				
Apr. 22, 1926	P	S	−	−	−	−				
May 21, 1926	+	P	−	−	−	−				
June 14, 1926	+	P	−	−	−	−				June 26, 1926, bred.
July 20, 1926	+	P	−	−	−	−				
Aug. 24, 1926	+	P	−	−	−	−				
Sept. 22, 1926	+	+	−	−	−	−				
Oct. 19, 1926	+	P	−	−	−	−				
Nov. 23, 1926	+	P	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	+	P	−	−	−	−				
Jan. 6, 1927	+	P	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	P	P	−	−	−	−				
Feb. 4, 1927	+	P	−	−	−	−				
Feb. 19, 1927	+	P	−	−	−	−				
Mar. 7, 1927	+	S	−	−	−	−				
Mar. 21, 1927	+	+	S	−	−	−				Mar. 21, 1927, expelled a living calf (268 days); 2 guinea pigs inoculated with uterine material and 4 with colostrum failed to acquire abortion disease.
Apr. 4, 1927	+	+	−	−	−	−				
Apr. 19, 1927	+	−	−	−	−	−				
May 2, 1927	P	−	−	−	−	−				
May 17, 1927	+	−	−	−	−	−				
June 1, 1927	+	−	−	−	−	−				
June 16, 1927	+	−	−	−	−	−				June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to contract abortion disease.
July 19, 1927	+	S	−	−	−	−				
Aug. 6, 1927	+	−	−	−	−	−				
Sept. 21, 1927	+	−	−	−	−	−				
Oct. 20, 1927	−	−	−	−	−	−				
Nov. 19, 1927	P	−	−	−	−	−				
Jan. 25, 1928	+	−	−	−	−	−				Apr. 2, 1928, bred.
June 6, 1928	P	−	−	−	−	−				
July 18, 1928	P	−	−	−	−	−				
Aug. 24, 1928	+	S	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	+	−	−	−	−	−				
Oct. 12, 1928	+	−	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	−	−	−	−	−	−				
Nov. 16, 1928	P	−	−	−	−	−				
Dec. 6, 1928	−	−	−	−	−	−				
Dec. 19, 1928	P	−	−	−	−	−				
Jan. 8, 1929	P	−	−	−	−	−				Jan. 3, 1929, expelled a living calf (276 days); 6 guinea pigs inoculated with placental emulsion and 6 with colostrum failed to contract abortion disease.
Jan. 28, 1929	−	−	−	−	−	−				
Feb. 28, 1929	P	−	−	−	−	−				
Apr. 11, 1929	P	−	−	−	−	−				

TABLE 5.—*Records of individual experiment animal*—Continued

CONTROL HEIFER 1269

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				
Oct. 4, 1925	−	−	−	−	−	−				
Nov. 10, 1925	−	−	−	−	−	−				
Dec. 10, 1925	−	−	−	−	−	−				
Jan. 15, 1926	−	−	−	−	−	−				
Feb. 15, 1926	−	−	−	−	−	−				
Mar. 16, 1926	−	−	−	−	−	−				
Apr. 22, 1926	−	−	−	−	−	−				
May 21, 1926	−	−	−	−	−	−				
June 14, 1926	−	−	−	−	−	−				June 20, 1926, bred.
July 20, 1926	−	−	−	−	−	−				July 8, 1926, bred.
Aug. 24, 1926	−	−	−	−	−	−				
Sept. 22, 1926	−	−	−	−	−	−				
Oct. 19, 1926	−	−	−	−	−	−				Oct. 23, 1926, bred.
Nov. 23, 1926	−	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	+	P	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 6, 1927	P	−	−	−	−	−				
Jan. 22, 1927	S	−	−	−	−	−				
Feb. 4, 1927	+	+	−	−	−	−				
Feb. 19, 1927	+	+	S	−	−	−				
Mar. 21, 1927	+	+	+	+	+	+				
Apr. 4, 1927	+	+	+	+	+	P	−	−	−	
Apr. 19, 1927	+	+	+	P	P	−				Apr. 14, 1927, expelled a dead fetus (173 days). *Bact. abortus* isolated from fetus and 4 guinea pigs inoculated with colostrum acquired abortion disease.
May 2, 1927	+	+	+	+	+	P				
May 17, 1927	+	+	+	+	−	−				
June 1, 1927	+	+	+	+	−	−				
June 16, 1927	+	+	+	+	P	P				June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk contracted abortion disease.
July 19, 1927	+	+	+	+	−	−				
Aug. 6, 1927	+	+	+	+	−	−				
Sept. 21, 1927	+	+	+	−	−	−				
Oct. 20, 1927	+	+	+	−	−	−				Oct. 20, 1927, 2 guinea pigs inoculated with a composite sample of milk acquired abortion disease.
Nov. 19, 1927	+	+	+	−	−	−				
Jan. 25, 1928	+	+	+	−	−	−				Jan. 25, 1928, 2 guinea pigs inoculated with a composite sample of milk acquired abortion disease.
Apr. 12, 1928	+	+	+	−	−	−				

CONTROL HEIFER 1275

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	P	S	−	−	−	−				
Sept. 2, 1925	S	−	−	−	−	−				
Oct. 4, 1925	−	−	−	−	−	−				
Nov. 10, 1925	−	−	−	−	−	−				
Dec. 10, 1925	−	−	−	−	−	−				
Jan. 15, 1926	−	−	−	−	−	−				
Feb. 15, 1926	−	−	−	−	−	−				
Mar. 16, 1926	−	−	−	−	−	−				
Apr. 22, 1926	+	−	−	−	−	−				
May 21, 1926	−	−	−	−	−	−				
June 14, 1926	−	−	−	−	−	−				June 20, 1926, bred.
July 20, 1926	−	−	−	−	−	−				
Aug. 24, 1926	−	−	−	−	−	−				
Sept. 22, 1926	−	−	−	−	−	−				
Oct. 19, 1926	−	−	−	−	−	−				
Nov. 23, 1926	−	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	−	−	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 6, 1927	−	−	−	−	−	−				
Jan. 22, 1927	P	−	−	−	−	−				
Feb. 4, 1927	−	−	−	−	−	−				
Feb. 9, 1927	−	−	−	−	−	−				
Mar. 7, 1927	−	−	−	−	−	−				

TABLE 5.—*Records of individual experiment animals*—Continued

CONTROL HEIFER 1275—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
Mar. 21, 1927	−	−	−	−	−	−				Mar. 26, 1927, expelled a living calf (279 days); 3 guinea pigs inoculated with uterine material and 4 with colostrum failed to contract abortion disease.
Apr. 4, 1927	−	−	−	−	−	−				
Apr. 19, 1927	−	−	−	−	−	−				
May 2, 1927	−	−	−	−	−	−				
May 17, 1927	+	+	+	+	−	−				May 21, 1927, 3 guinea pigs inoculated with a composite sample of milk failed to contract abortion disease.
June 1, 1927	+	+	+	P	−	−				
June 16, 1927	+	P	−	−	−	−				June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to acquire abortion disease.
July 13, 1927	+	−	−	−	−	−				
Aug. 6, 1927	+	+	−	−	−	−				
Sept. 21, 1927	P	−	−	−	−	−				
Oct. 20, 1927	−	−	−	−	−	−				
Nov. 19, 1927	−	−	−	−	−	−				
Jan. 25, 1928	+	−	−	−	−	−				

CONTROL HEIFER 1280

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	+	P	−	−	−	−				
July 29, 1925	+	S	−	−	−	−				
Sept. 2, 1925	+	P	−	−	−	−				
Oct. 4, 1925	+	P	−	−	−	−				
Nov. 10, 1925	+	P	−	−	−	−				
Dec. 10, 1925	+	−	−	−	−	−				
Jan. 15, 1926	S	−	−	−	−	−				
Feb. 15, 1926	−	−	−	−	−	−				
Mar. 16, 1926	P	−	−	−	−	−				
Apr. 22, 1926	+	−	−	−	−	−				
May 21, 1926	S	−	−	−	−	−				
June 14, 1926	+	P	−	−	−	−				June 22, 1926, bred.
July 20, 1926	+	S	−	−	−	−				
Aug. 24, 1926	+	S	−	−	−	−				
Sept. 22, 1926	P	S	−	−	−	−				
Oct. 19, 1926	+	−	−	−	−	−				
Nov. 23, 1926	+	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	−	−	−	−	−	−				
Jan. 6, 1927	P	S	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	P	P	−	−	−	−				
Feb. 4, 1927	+	−	−	−	−	−				
Feb. 19, 1927	+	+	−	−	−	−				
Mar. 7, 1927	+	+	−	−	−	−				
Mar. 21, 1927	+	S	−	−	−	−				
Apr. 4, 1927	+	+	−	−	−	−				Apr. 1, 1927, expelled a living calf (283 days); 4 guinea pigs inoculated with uterine material and 4 with colostrum failed to acquire abortion disease.
Apr. 19, 1927	+	−	−	−	−	−				
May 2, 1927	+	−	−	−	−	−				
May 17, 1927	+	−	−	−	−	−				
June 1, 1927	+	−	−	−	−	−				
June 16, 1927	+	−	−	−	−	−				
July 19, 1927	+	+	−	−	−	−				
Aug. 6, 1927	+	−	−	−	−	−				
Sept. 21, 1927	−	−	−	−	−	−				
Nov. 19, 1927	+	−	−	−	−	−				
Jan. 25, 1928	+	−	−	−	−	−				

CONTROL HEIFER 1303

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	S	−	−	−	−	−				
Sept. 2, 1925	P	−	−	−	−	−				
Oct. 4, 1925	−	−	−	−	−	−				
Nov. 10, 1925	−	−	−	−	−	−				
Dec. 10, 1925	−	−	−	−	−	−				
Jan. 15, 1926	−	−	−	−	−	−				
Feb. 15, 1926	−	−	−	−	−	−				
Mar. 16, 1926	−	−	−	−	−	−				
Apr. 22, 1926	−	−	−	−	−	−				

TABLE 5.—*Records of individual experiment animals*—Continued

CONTROL HEIFER 1303—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
May 21, 1926	−	−	−	−	−	−				
June 14, 1926	−	−	−	−	−	−				
July 20, 1926	−	−	−	−	−	−				July 3, 1926, bred.
Aug. 24, 1926	−	−	−	−	−	−				
Sept. 22, 1926	−	−	−	−	−	−				
Oct. 19, 1926	−	−	−	−	−	−				
Nov. 23, 1926	−	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	−	−	−	−	−	−				
Jan. 6, 1927	−	−	−	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	−	−	−	−	−	−				
Feb. 4, 1927	+	−	−	−	−	−				Feb. 3, 1927, expelled a dead fetus (215 days); *Bact. abortus* isolated from fetus; 4 guinea pigs inoculated with colostrum contracted abortion disease.
Feb. 19, 1927	P	−	−	−	−	−				
Mar. 7, 1927	+	+	−	−	−	−				
Mar. 21, 1927	+	+	+	+	−	−				
Apr. 4, 1927	+	+	+	+	+	−				
Apr. 19, 1927	+	+	+	+	P	−				
May 2, 1927	+	+	+	+	−	−				
May 17, 1927	+	+	+	+	−	−				
June 1, 1927	+	+	+	+	−	−				
June 16, 1927	+	+	+	+	−	−				June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk failed to contract abortion disease.
July 19, 1927	+	+	+	+	−	−				
Aug. 6, 1927	+	+	+	+	+	+				
Sept. 21, 1927	+	+	+	+	+	+				
Oct. 20, 1927	+	+	+	+	+	+	+	−	−	Oct. 20, 1927, 2 guinea pigs inoculated with a composite sample of milk contracted abortion disease.
Nov. 19, 1927	+	+	+	+	+	+	−			
Jan. 25, 1928	+	+	+	+	+	+	+	+	−	

CONTROL HEIFER 1304

May 21, 1925	−	−	−	−	−	−				
June 26, 1925	−	−	−	−	−	−				
July 29, 1925	−	−	−	−	−	−				
Sept. 2, 1925	−	−	−	−	−	−				
Oct. 4, 1925	−	−	−	−	−	−				
Nov. 10, 1925	−	−	−	−	−	−				
Dec. 10, 1925	−	−	−	−	−	−				
Jan. 15, 1926	−	−	−	−	−	−				
Feb. 15, 1926	−	−	−	−	−	−				
Mar. 16, 1926	−	−	−	−	−	−				
Apr. 22, 1926	−	−	−	−	−	−				
May 21, 1926	−	−	−	−	−	−				
June 14, 1926	−	−	−	−	−	−				June 27, 1926, bred.
July 20, 1926	−	−	−	−	−	−				
Aug. 24, 1926	−	−	−	−	−	−				
Sept. 22, 1926	−	−	−	−	−	−				
Oct. 19, 1926	−	−	−	−	−	−				
Nov. 23, 1926	−	−	−	−	−	−				Nov. 26, 1926, received 150 c. c. emulsion of fetal stomach and intestinal contents.
Dec. 18, 1926	+	+	−	−	−	−				
Jan. 6, 1927	+	+	P	−	−	−				Nov. 29, 1926, received 125 c. c. emulsion of fetal stomach and intestinal contents.
Jan. 22, 1927	+	+	+	+	S	−				
Feb. 4, 1927	+	+	+	+	+	+	−			
Feb. 19, 1927	+	+	+	+	+	+	+	+	−	Feb. 18, 1927, expelled a dead fetus (236 days); *Bact. abortus* isolated from fetus; 5 guinea pigs inoculated with colostrum contracted abortion disease.
May 2, 1927	+	+	+	+	+	+	+	+	+	
May 17, 1927	+	+	+	+	+	+	+	+	+	
June 1, 1927	+	+	+	+	+	+	+	+	+	
June 16, 1927	+	+	+	+	+	+	+	+	+	June 15, 1927, 2 guinea pigs inoculated with a composite sample of milk contracted abortion disease.
July 19, 1927	+	+	+	+	+	+	+	+	−	
Aug. 6, 1927	+	+	+	+	+	+	+	−		
Sept. 21, 1927	+	+	+	+	P	−				
Oct. 20, 1927	+	+	+	+	+	−				Oct. 20, 1927, 2 guinea pigs inoculated with a composite sample of milk contracted abortion disease.
Nov. 19, 1927	+	+	+	+	+	+	−			
Jan. 25, 1928	+	+	+	+	+	+	−			

TABLE 5.—*Records of individual experiment animals*—Continued

CONTROL COW 1360

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
June 2, 1928	−	−	−	−	−	−				Feb. 6, 1928, bred.
July 18, 1928	−	−	−	−	−	−				
Aug. 24, 1928	−	−	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	−	−	−	−	−	−				
Oct. 12, 1928	−	−	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	−	−	−	−	−	−				
Nov. 16, 1928	−	−	−	−	−	−				Nov. 21, 1928, expelled a living calf (288 days); 6 guinea pigs inoculated with placental material and 5 with colostrum failed to contract abortion disease.
Dec. 6, 1928	−	−	−	−	−	−				
Dec. 19, 1928	−	−	−	−	−	−				
Jan. 8, 1929	−	−	−	−	−	−				
Jan. 28, 1929	−	−	−	−	−	−				

CONTROL HEIFER 1419

June 2, 1928	−	−	−	−	−	−				Apr. 3, 1928, bred.
June 6, 1928	−	−	−	−	−	−				
July 18, 1928	−	−	−	−	−	−				
Aug. 24, 1928	−	−	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	−	−	−	−	−	−				
Oct. 12, 1928	−	−	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	−	−	−	−	−	−				
Nov. 16, 1928	−	−	−	−	−	−				
Dec. 6, 1928	−	−	−	−	−	−				
Dec. 19, 1928	−	−	−	−	−	−				Dec. 28, 1928, expelled a dead calf (269 days); 8 guinea pigs inoculated with uterine material and 4 with colostrum failed to acquire abortion disease.
Jan. 8, 1929	−	−	−	−	−	−				
Jan. 28, 1929	−	−	−	−	−	−				

CONTROL HEIFER 1425

June 2, 1928	−	−	−	−	−	−				Apr. 4, 1928, bred.
July 18, 1928	−	−	−	−	−	−				
Aug. 24, 1928	−	−	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	+	+	+	+	S	−				
Oct. 12, 1928	+	+	+	+	P	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	+	+	+	+	+	−				
Nov. 16, 1928	+	+	+	+	P	−				
Dec. 6, 1928	+	+	+	+	−	−				
Dec. 19, 1928	+	+	+	+	+	−				Dec. 18, 1928, expelled a living calf (258 days); 6 guinea pigs inoculated with placental material and 6 with colostrum contracted abortion disease.
Jan. 8, 1929	+	+	+	+	+	+				
Jan. 28, 1929	+	+	+	+	+	+				

CONTROL HEIFER 1426

June 2, 1928	−	−	−	−	−	−				Mar. 24, 1928, bred.
July 18, 1928	−	−	−	−	−	−				Apr. 14, 1928, bred.
Aug. 24, 1928	P	−	−	−	−	−				Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	+	P	−	−	−	−				
Oct. 12, 1928	+	S	−	−	−	−				Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 27, 1928	+	+	+	+	+	−				
Nov. 16, 1928	+	+	+	+	+	+				Nov. 18, 1928, expelled a dead fetus (218 days); *Bact. abortus* isolated from fetus; 5 guinea pigs inoculated with colostrum contracted abortion disease.
Dec. 6, 1928	+	+	+	+	+	+				
Dec. 19, 1928	+	+	+	+	+	+				
Jan. 8, 1929	+	+	+	+	+	+				
Jan. 28, 1929	+	+	+	+	+	+				

TABLE 5.—*Records of individual experiment animals*—Continued

CONTROL HEIFER 1453

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used									Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002	0.0001	
July 18, 1928	—	—	—	—	—	—	-----	-----	-----	Breeding date not recorded.
Aug. 24, 1928	—	—	—	—	—	—	-----	-----	-----	Aug. 3, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Sept. 19, 1928	—	—	—	—	—	—	-----	-----	-----	Aug. 9, 1928, received 100 c. c. emulsion of fetal intestinal contents.
Oct. 12, 1928	—	—	—	—	—	—	-----	-----	-----	Aug. 28, 1928, expelled a seemingly normal calf; 5 guinea pigs inoculated with placental material and 4 with colostrum failed to contract abortion disease.
Oct. 27, 1928	—	—	—	—	—	—	-----	-----	-----	
Nov. 16, 1928	—	—	—	—	—	—	-----	-----	-----	
Dec. 6, 1928	—	—	—	—	—	—	-----	-----	-----	
Dec. 19, 1928	—	—	—	—	—	—	-----	-----	-----	
Jan. 8, 1929	—	—	—	—	—	—	-----	-----	-----	
Jan. 28, 1929	—	—	—	—	—	—	-----	-----	-----	

DISCUSSION OF RESULTS

The three heifers receiving the vaccine of supposedly medium virulence (*Bacterium abortus* strain 19) gave evidence of not only resisting the localization of the infection in their bodies but of successfully withstanding the degree of *Bact. abortus* exposure to which they were subsequently subjected. One of the three heifers which received the vaccine that was regarded as most virulent (*Bact. abortus* strain 88) appeared to be rendered a more or less permanent carrier of the infection in the udder, and one of the five heifers receiving vaccine prepared from strains 8, 10, and 11 that had been under artificial cultivation longest failed to resist the invasion of the disease when later exposed. The limited data accumulated with reference to the proper virulence of *Bact. abortus* strains for calfhood vaccination, therefore, suggested that strains of medium virulence might be superior either to those of prolonged artificial cultivation or to those of very recent isolation.

The breeding records of the animals used in the experiment failed to suggest that the use of the vaccine induced sterility, for only on rare occasions, as is indicated by the individual records, did either the principals or the controls fail to conceive on the first breeding.

While 2 of the 11 vaccinated animals eliminated *Bacterium abortus* in their colostrum either during their first parturition, or second, or both, Tables 3 and 4 show that 5 of the 10 controls from similar *Bact. abortus* exposure also became eliminators of the infection through this channel at times of calving or aborting. Since udder infection of but one principal could in fairness seemingly be charged to the vaccine injection, and since the most recently isolated strain of *Bact. abortus* was used in the preparation of the vaccine which this particular animal received, the method was not regarded as being seriously objectionable in this respect. The results of the experiment were, on the whole, encouraging.

CONCLUSIONS

The information which this calfhood-immunization experiment yielded leads to the following conclusions:

It is possible, by the subcutaneous administration of abortion vaccine during calfhood, to engender in bovines an immunity to

Bacterium abortus infection that is readily demonstrable during their first pregnancies.

The immunity afforded by early vaccination, possibly somewhat reinforced by *Bacterium abortus* ingestion exposures, seems to continue through second gestation.

Vaccine prepared with a *Bacterium abortus* strain of medium virulence may be preferable to those of very recent isolation or those of long-continued propagation on artificial media for calfhood-immunization purposes.

Vaccination during calfhood does not appear to produce effects that are manifested as sterility when the animals arrive at breeding age.

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THE CATALASE CONTENT OF THE COLORADO POTATO BEETLE DURING METAMORPHOSIS [1]

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INTRODUCTION

In a previous paper, dealing with respiratory metabolism during the embryonic and metamorphic development of insects, the writer (*5*) [3] pointed out several striking results in the gaseous exchange obtained during prepupal and pupal development. From an analysis of the data shown in Figures 6 to 10, inclusive, of that article, the inference was drawn that the curves represented the rate of metabolism during histolysis and pupal differentiation. That such an inference was justified is apparent from the marked diminution in the rate of gaseous exchange during the first few days of the prepupal condition, as illustrated by the descending phase of the curve and by the gradual increase in the rate of oxygen consumption and carbon-dioxide output during the last days of the prepupal period, as illustrated by the ascending phase of the curve.

In view of these investigations, it seemed to the writer a matter of considerable interest to determine whether other physiological phenomena of insects were markedly influenced during metamorphosis. An attempt was therefore made to observe the changes in the catalase content of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) during its histolysis and pupal differentiation.

REVIEW OF LITERATURE

There are no records in the literature which indicate determinations of catalase during the complete metamorphosis of an insect. Although Burge and Burge (*4*) investigated the catalase content of the potato beetle in different stages, no attempt was made by these writers to trace the course of activity of catalase during metamorphosis.

Other investigators have sought by experiment to determine the influence of certain hormones on the course of metamorphosis. Thus, Kopeć (*9*), in one of a series of papers on the metamorphosis of insects, stated that the administration of thyroid extracts to caterpillars of *Lymantria dispar* did not cause any distinct changes in the duration of the larval or pupal period. From other experiments performed on caterpillars of the gipsy moth, Kopeć (*7*, *8*) concluded that probably, through the agency of internal secretion, the brain had the separate functions in hormonal action of causing histolysis of larval tissue and retarding the development of imaginal disks.

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[2] During the summer of 1927 the writer was assisted by I. R. Taylor and C. E. Herber, field assistants, in the experimental work described in this paper.

[3] Reference is made by number (italic) to Literature Cited, p. 695.

Tiegs (*12*) holds that metamorphosis is brought about by a widespread death of tissue, due to an automatic starvation of larval cells. He argues that the cell contents, which increase as the cube of the radius of the growing cell, can not be nourished indefinitely through the cell membrane, the area of which increases only as the square of the radius. Bataillon (*1*), as the result of certain experiments on silkworms, decided that an accumulation of carbon dioxide in the tissues was an attendant factor in metamorphosis. This theory, however, has been disproved by the recent experiments of Singh-Pruthi (*11*), who placed larvae in a saturated atmosphere of carbon dioxide and found that this gas did not hasten metamorphosis but markedly retarded it.

MATERIAL AND METHODS

To observe the changes in catalase content during the histolysis, histogenesis, and pupal differentiation of the potato beetle, determinations of oxygen liberated from hydrogen peroxide by catalase were made with an apparatus essentially similar to the one described by Burge (*3*) in his research on the effect of work on catalase of muscle. Two inverted burettes, each of 50 c. c. capacity, were held in place by clamps and iron stands, the mouths of the burettes being set in a shallow pan of water and connected by means of glass tubing to a small flask in which oxygen was generated. By means of suitable screw clamps the flow of oxygen liberated by the action of catalase upon H_2O_2 in the generating flask could be diverted to one or the other of the two inverted burettes, and the volume of oxygen obtained by displacement of water in a given time was read on the scales of the burettes. The volumes of gas were reduced to standard conditions of temperature and pressure.

In view of the work of Morgulis, Beber, and Rabkin (*10*) and others, who have determined that destruction of catalase may occur when an excess of H_2O_2 is present, it seemed necessary to determine by preliminary experiment the minimum quantity of H_2O_2 to use in the investigations on the potato beetle. By preliminary trials with single larvae and pupae during 20-minute periods starting with various concentrations of superoxol from 0.5 to 5 per cent, and in other tests by also gradually increasing the quantity of superoxol solution from 10 to 30 cubic centimeters, it was found that at the end of 20 minutes no further oxygen was evolved when 30 cubic centimeters of a 3 per cent superoxol was used. This quantity was therefore adopted in all of the experiments with the potato beetle.

Mature, or nearly mature, larvae of the potato beetle, collected in the field, were brought into the laboratory and isolated in 1-ounce tin salve boxes containing damp sand in which the larvae might bury themselves when ready to pass through the prepupal period and transform to the pupa. The larvae in the salve boxes were kept at a temperature of 22° to 25° C. and examined twice daily, and a record was kept of the duration of the prepupal stage, the exact date of the formation of the pupa, and the development of the pupa.

In the experimental procedure for determination of catalase, a single larva was first weighed to the nearest milligram, ground in a mortar with a definite quantity of clean sand (washed and dried), and immediately transferred to the stoppered generating flask of the

apparatus already described; to the mixture was added 30 cubic centimeters of a 3 per cent solution of superoxol, previously adjusted to pH 7.0. The evolution of oxygen was observed as has been described.

Determinations of catalase were first made on mature larvae before they entered the soil for prepupation, and on material of each day of development thereafter throughout the prepupal and pupal stages. The experiments were conducted on three series of larvae collected during July and August. A series consisted of determinations of the oxygen liberated for each day of development, commencing with the mature larva, and extending through the prepupal and pupal periods. At least 10 determinations, conducted at room temperature, were made each day during the development of the insects in the series. In each determination the evolution of oxygen was limited to 20 minutes, the volume of gas collected being measured by successive readings for each of four consecutive 5-minute periods.

TABLE 1.—*Oxygen liberated from hydrogen peroxide by the catalase of the Colorado potato beetle, at intervals of one day, during prepupal and pupal development*

Stage of insect	Range of temperature during experiments	Insects	Average weight of insect	Average oxygen liberated per gram of insect in each of 4 consecutive 5-minute periods				Total oxygen liberated per gram in 20 minutes
				First period	Second period	Third period	Fourth period	
	°C.	Number	Gram	Cu. cm.	Cu. cm.	Cu. cm.	Cu. cm.	Cu. cm.
Mature larva	24–26	32	0.167	733.7	470.3	230.4	110.2	1,547.6
Prepupa:								
First day	23 –25	36	.147	586.1	346.1	223.1	143.7	1,299.0
Second day	23.6–25.6	37	.139	570.2	322.2	226.7	134.7	1,253.8
Third day	22 –24.4	34	.150	600.4	427.9	313.9	192.7	1,534.9
Pupa (when formed)	21.6–23.6	31	.145	632.2	439.8	343.7	232.1	1,647.8
First day	22.6–24.6	30	.156	567.5	419.1	329.8	191.9	1,508.3
Second day	22.8–24.2	27	.148	571.5	412.1	331.6	205.5	1,520.7
Third day	23 –25	40	.145	507.4	343.2	283.4	208.0	1,342.0
Fourth day	23.8–25.2	28	.141	439.7	304.7	268.3	212.3	1,225.0
Fifth day	23.5–24.5	26	.147	390.8	256.5	231.7	154.0	1,033.0
Sixth day	23.8–25.2	21	.148	293.5	193.6	148.7	117.3	753.1

EXPERIMENTAL DATA

To indicate the relative catalase content for each day of development of the insect, data from three complete series were averaged. Table 1 presents for each day of development the average volume, in cubic centimeters per gram of insect material used, of oxygen liberated in each of the 5-minute periods, and the total of these averages for the entire 20 minutes.

Striking differences in the proportion of catalase present at successive stages of development are suggested by the varying quantities of oxygen evolved per gram, as shown in each of the five columns of results. The quantities for the four 5-minute periods are presented graphically by the lower four curves in Figure 1, the totals for the 20-minute interval by the top curve. The most pronounced inhibition of activity of catalase was observed to occur on the first and second days of the prepupal period. As compared with the similar activity in the mature larva, the oxygen liberated in the 20-minute period on the first day of the prepupal stage was 16 per cent less, and

on the second day it was at its lowest recorded point of 19 per cent less. On the third and final day of the prepupal stage it was only 0.8 per cent less. On the following day, with the pupa formed, the evolution of oxygen in the 20-minute period reached its maximum, being 6 per cent greater than that for the mature larva, and exceeding it for the only time. Thereafter a steady decline was recorded until the lowest liberation of oxygen was obtained with the sixth day of the pupa

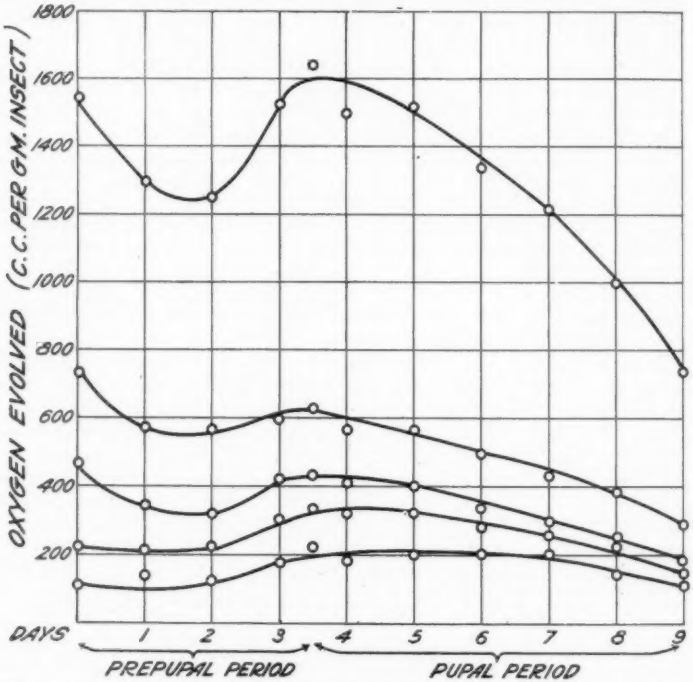

FIGURE 1.—Average quantities of oxygen evolved from hydrogen peroxide by the action of catalase, in the Colorado potato beetle, for each day during the prepupal and pupal stages. The top curve shows the total oxygen produced during 20 minutes for each day of development. The four lower curves, in order, beginning at the top, indicate the average evolution of oxygen in four consecutive 5-minute periods, the sums of the ordinates for each day being the average total oxygen produced in the 20 minutes allowed for each individual determination

(formation of the adult), a reduction of 51 per cent from the rate for the mature larva.

Although the type of curve indicated for the oxygen released by catalase during histolysis very closely resembles the curve for CO_2 also evolved during histolysis, it is quite evident from the subsequent divergence of the former curve from that of the CO_2 during pupal development that they do not measure the same reaction. Burge and Burge (*4*) attributed the increase in the rate of oxidation in youth and its decrease in old age to the increase and decrease in catalase in the respective cases. Bodine (*2*) found that in the case of certain insects the content of catalase decreases with increasing age and with starvation, and that in hibernation there is a marked decrease in the

output of CO_2 with no corresponding change in catalase content, and Fink (*6*) found a correlation between the reduced activity of catalase and diminished respiratory metabolism in starving and hibernating insects, and that catalase activity of hibernating beetles is greatly reduced and their total catalase content is lower than that of old or young active beetles. It is agreed among physiologists that increased excretion of CO_2 may be considered a satisfactory index of development, since that excretion is generally accepted as an index of metabolizing tissue. The excretion of CO_2 is also a far better index of growth than increase in weight, since the latter may also be due to an increased content of inert or nonliving substances. Catalase content, however, is not an index of growth or development, since the curve produced by its action on hydrogen peroxide does not parallel that of CO_2 during the entire development, although it approximately does so during histolysis. The content of catalase seems to decrease during the pupal development, whereas the excretion of CO_2 increases.

The extensive disruption of tissue during histolysis may account partly for the decreased content of catalase manifested during the first few days of the prepupal period. If, as pointed out by other investigators, the function of the catalase is to prevent the formation of hydrogen peroxide in the living tissue, the low catalase content usually measured during histolysis might indicate that during this period an exceptionally large supply of catalase was required by unhistolyzed tissue to neutralize the excess peroxide formed during the process of histolysis, and that the catalase found at this time was simply that remaining after the dissociation of the peroxide in the living tissue.

SUMMARY

The variation in the activity of catalase during the metamorphosis of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), beginning with the mature larva, was ascertained by making daily determinations of the quantity of oxygen per gram of tissue evolved from hydrogen peroxide by the action of catalase during prepupal and pupal development.

Analysis of the resulting data indicated a reduction in catalase of 16 per cent on the first day of histolysis and 19 per cent on the second, followed on the third day of histolysis by an increase which, when the pupa was formed, reached the maximum of 6 per cent above that of the mature larve. The decline in content of catalase during pupal development was continuous thereafter until a reduction of 51 per cent below that of the mature larva was reached with the formation of the adult.

The curves here obtained for catalase content and those previously reported by the writer for oxygen consumption and excretion of carbon dioxide (*5*) were similar for the prepupal period of histolysis. During pupal development, however, the catalase curve declined rapidly, whereas the curves for oxygen and carbon dioxide followed the usual growth curve.

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